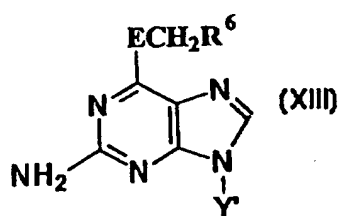
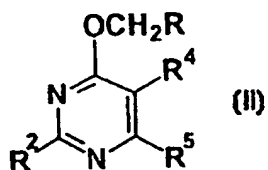




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| <p>(21) International Application Number: PCT/IE96/00084</p> <p>(22) International Filing Date: 9 December 1996 (09.12.96)</p> <p>(30) Priority Data: 08/568,576 7 December 1995 (07.12.95) US 08/572,966 15 December 1995 (15.12.95) US</p> <p>(71) Applicant (for all designated States except US): CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; Cambridge House, 6-10 Cambridge Terrace, Regent's Park, London NW1 4JL (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only): McMURRY, Thomas, Brian, Hamilton [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). McELHINNEY, Robert, Stanley [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). McCORMICK, Joan, Elizabeth [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). DONNELLY, Dorothy, Josephine [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). MURRAY, Paul [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). CAROLA, Christophe [FR/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). ELDER, Rhoderick, Hugh [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). KELLY, Jane [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). MARGISON, Geoffrey, Paul [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). WATSON, Amanda, Jean [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). RAFFERTY, Joseph, Anthony [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). WILLINGTON, Mark, Andrew [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). MIDDLETON, Mark, Ross [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB).</p> <p>(74) Agents: PARKES, Andrew, John, Aykroyd et al.; Tomkins & Co., 5 Dartmouth Road, Dublin 6 (IE).</p> <p>(81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p> | | |

(54) Title: PYRIMIDINE DERIVATIVES AND GUANINE DERIVATIVES, AND THEIR USE IN TREATING TUMOUR CELLS



(57) Abstract

The invention provides compounds exhibiting the ability to deplete O⁶-alkylguanine-DNA alkyltransferase (ATase) activity in tumour cells. The compounds include certain pyrimidine derivatives of formula (II), wherein R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N, or S, or a substituted derivative thereof; or (ii) phenyl or a substituted derivative thereof, R² is selected from H, C¹-C⁵ alkyl, halogen or NH², R⁴ and R⁵ which are the same or different are selected from H, NH-Y' or NO_n wherein Y' is H, ribosyl, deoxyribosyl, arabinosyl, (a) wherein X is O or S, R'' is alkyl and R''' is H or alkyl, or substituted derivatives thereof, n = 1 or 2 or R⁴ and R⁵ together with the pyrimidine ring form a 5- or 6-membered ring structure containing one or more hetero atoms, and pharmaceutically acceptable salts thereof. They include certain guanine derivatives of formula (XIII), wherein R⁶ is as defined at (i) for R above and Y' is as defined above.

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PYRIMIDINE DERIVATIVES AND GUANINE DERIVATIVES, AND THEIR USE IN
TREATING TUMOUR CELLS

15 Technical Field

The present invention relates to pyrimidine derivatives and
guanine derivatives, and their use in treating tumour cells. In
particular, it relates to 6-hetarylalkyloxy pyrimidine derivatives,
20 \underline{Q}^6 -substituted guanine derivatives and \underline{S}^6 -substituted
thioguanine derivatives, these compounds exhibiting the ability to
deplete \underline{Q}^6 -alkylguanine-DNA alkyltransferase (ATase) activity in
tumour cells.

25 Background Art

It has been suggested to use \underline{Q}^6 -alkyl guanine derivatives
possessing \underline{Q}^6 -alkylguanine-DNA alkyltransferase depleting activity
in order to enhance the effectiveness of chemotherapeutic alkylating
30 agents, principally those that methylate or chloroethylate DNA, used
for killing tumour cells. There is increasing evidence that in
mammalian cells the toxic and mutagenic effects of alkylating agents
are to a large extent a consequence of alkylation at the
 \underline{Q}^6 -position of guanine in DNA. The repair of \underline{Q}^6 -alkylguanine is
35 mediated by ATase, a repair protein that acts on the \underline{Q}^6 -alkylated
guanine residues by stoichiometric transfer of the alkyl group to a
cysteine residue at the active site of the repair protein in an

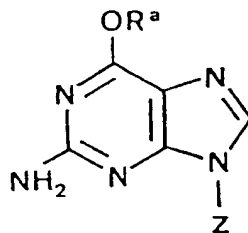
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autoinactivating process. The importance of ATase in protecting cells against the biological effects of alkylating agents has been most clearly demonstrated by the transfer and expression of cloned
 5 ATase genes or cDNAs into ATase deficient cells: this confers resistance to a variety of agents, principally those that methylate or chloroethylate DNA. Whilst details of the mechanism of cell killing by O^6 -methylguanine in ATase deficient cells is not yet clear, killing by O^6 -chloroethylguanine occurs through DNA
 10 interstrand crosslink formation to a cytosine residue on the opposite strand via a cyclic ethanoguanine intermediate, a process that is prevented by ATase-mediated chloroethyl group removal or complex formation.

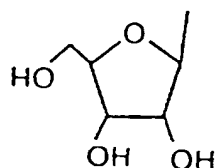
15 The use of O^6 -methylguanine and O^6 -n-butylguanine for depleting ATase activity has been investigated (Dolan *et al.*, Cancer Res., (1986) 46, pp. 4500; Dolan *et al.*, Cancer Chemother. Pharmacol., (1989) 25, pp 103. O^6 -benzylguanine derivatives have been proposed for depleting ATase activity in order to render
 20 ATase expressing cells more susceptible to the cytotoxic effects of chloroethylating agents (Moschel *et al.*, J. Med.Chem., 1992, 35, 4486). U.S. Patent 5 091 430 and International Patent Application No. WO 91/13898 Moschel *et al.* disclose a method for depleting levels of O^6 -alkylguanine-DNA alkyl-transferase in tumour cells in
 25 a host which comprises administering to the host an effective amount of a composition containing O^6 -benzylated guanine derivatives of the following formula:

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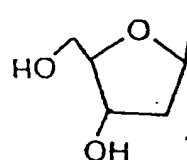
wherein Z is hydrogen, or



35



or

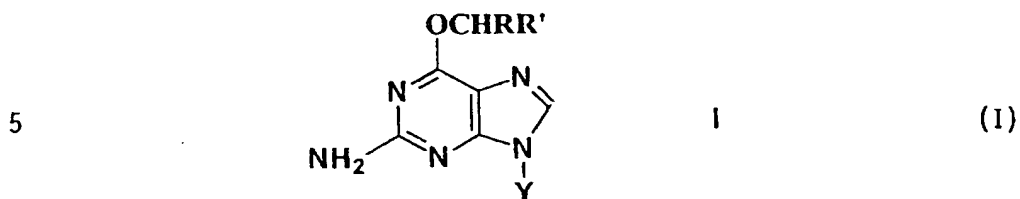


- 3 -

and R^a is a benzyl group or a substituted benzyl group. A benzyl group may be substituted at the ortho, meta or para position with a substituent group such as halogen, nitro, aryl such as phenyl or substituted phenyl, alkyl of 1-4 carbon atoms, alkoxy of 1-4 carbon atoms, alkenyl of up to 4 carbon atoms, alkynyl of up to 4 carbon atoms, amino, monoalkylamino, dialkylamino, trifluoromethyl, hydroxy, hydroxymethyl, and SO_nR^b wherein n is 0, 1, 2 or 3 and R^b is hydrogen, alkyl of 1-4 carbon atoms or aryl. Chae et al., J. Med. Chem., 1994, 37, 342-347 describes tests on Q⁶-benzylguanine analogs bearing increasingly bulky substituent groups on the benzene ring or at position 9. Chae et al., J. Med. Chem. 1995, 38, 359-365 describe several 8-substituted Q⁶-benzylguanines, 2- and/or 8-substituted 6-(benzyloxy)purines, substituted 6(4)-(benzyloxy)pyrimidines, and a 6-(benzyloxy)-s-triazine which were tested for their ability to inactivate ATase. Two types of compounds were identified as being significantly more effective than Q⁶-benzylguanine at inactivating ATase in human HT29 colon tumour cell extracts. These were 8-substituted Q⁶-benzylguanines bearing electron-withdrawing groups at the 8-position (e.g. 8-aza-Q⁶-benzylguanine and Q⁶-benzyl-8-bromoguanine) and 5-substituted 2,4-diamino-6-(benzyloxy)pyrimidines bearing electron withdrawing groups at the 5-position (e.g. 2,4-diamino -6-(benzyloxy)-5-nitroso- and 2,4-diamino-6-(benzyloxy)-5-nitropyrimidine). The latter derivatives were also more effective than Q⁶-benzylguanine at inactivating ATase in intact HT29 colon tumour cells. WO 96/04280 published after the priority dates of this application concerns similar substituted Q⁶-benzylguanines and 6(4)-benzyloxypyrimidines.

The present Applicants are also Applicants in International Patent Application PCT/IE94/00031 which was published under No. WO 94/29312. WO 94/29312 (the contents of which are incorporated herein by reference in their entirety) describes Q⁶-substituted guanine derivatives of formula I:

- 4 -



wherein

10 Y is H, ribosyl, deoxyribosyl, or R''XCHR''',
 wherein X is O or S, R'' and R''' are alkyl, or
 substituted derivatives thereof;

R' is H, alkyl or hydroxyalkyl;

15 R is (i) a cyclic group having at least one 5- or 6-membered
 heterocyclic ring, optionally with a carbocyclic or heterocyclic
 ring fused thereto, the or each heterocyclic ring having at least
 one hetero atom chosen from O, N, or S, or a substituted derivative
 thereof; or

20 (ii) naphthyl or a substituted derivative thereof;

and pharmaceutically acceptable salts thereof.

25 In order to be useful for depleting ATase activity and thus
 enhance the effects of the above-mentioned chemotherapeutic agents,
 compounds should have combination of characteristics assessed by
 reference to:

30 1) *In vitro* inactivation of recombinant ATases.

2) Stability.

3) Solubility.

35

4) Inactivation of ATase in mammalian cells and/or tumour
 xenografts.

- 5 -

5) Sensitization of mammalian cells and/or tumour xenografts to the killing or growth inhibitory effects of the said chemotherapeutic agents

5

The behaviour of novel compounds in this combination of tests is unpredictable. Molecular interactions including steric factors in the unpredictability of ATase inactivation may be related to the nature of the environment of the cysteine acceptor site in the ATase molecule.

10

The structure of the ATase protein derived from *E. coli* (Ada gene) has been elucidated by X-ray crystallographic techniques (M.H. Moore *et. al.*, *EMBO Journal*, 1994, 13, 1495.). While the amino acid sequence of human ATase differs somewhat from that of bacterial origin, all known ATases (human, rodent, yeast, bacterial) contain the cysteine (Cys) acceptor site in a common fragment, Pro-Cys-His-Arg. A homology model of human ATase generated by computer from the crystal structure of the Ada protein (J.E.A. Wibley *et. al.*, *Anti-Cancer Drug Design*, 1995, 10, 75.) resembles it in having the Cys acceptor buried in a pocket deep in the protein. Considerable distortion of the structure is necessary to bring either an O^6 -alkylated guanine residue in intact DNA, or even free guanine alkylated by a relatively large group like benzyl, close to the Cys acceptor. These configurational changes are initiated by a characteristic binding of duplex DNA to the protein (K. Goodtzova *et. al.* *Biochemistry*, 1994, 33, 8385).

Since the amino acid components and dimensions of the ATase active site "pocket" are still unknown as are the details of the mechanism involved, it is impossible to predict the activity of a particular O^6 -alkylated guanine or analogous ring system.

Published work in this field relates predominantly to the use of O^6 -alkyl guanine derivatives having a nucleus identical to that of guanine in DNA. Chae *et. al.*, *J. Med. Chem.* 1995, 38, 359-365 have described tests on a limited number of compounds in which the

35

- 6 -

guanine ring was modified. However these compounds all had benzyl substitution at the O^6 - position of the modified guanine ring or 6(4)-benzyloxy substitution on the pyrimidine ring. The observation that subtle changes in the substituents on the guanine ring or in the purine skeleton can generate agents that are very ineffective ATase inactivators, in comparison with their "parent" structure, suggests that more substantial modifications might also disrupt the ATase inactivating function.

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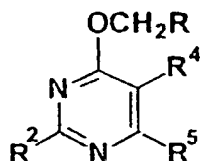
There is a need for additional novel compounds useful for depleting ATase activity in order to enhance the effects of chemotherapeutic agents such as chloroethylating or methylating anti-tumour agents. It is a further object to provide compounds having better ATase inactivating characteristics than O^6 -benzylguanine and having different solubility patterns.

Another object of the invention is to provide pharmaceutical compositions containing compounds which are useful for depleting ATase activity. A further object of the present invention is to provide a method for depleting ATase activity in tumour cells. A still further object of the invention is to provide a method for treating tumour cells in a host in such a way that they become more sensitive to the above-mentioned alkylating agents.

25

The present invention provides 6-hetarylalkyloxy pyrimidine derivatives of formula II:

30



II

wherein

35

R is (i) a cyclic group having at least one 5- or 6- membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring

- 7 -

having at least one hetero atom chosen from O, N or S, or a substituted derivative thereof; or

(ii) phenyl or a substituted derivative thereof,

- 5 R^2 is selected from H, C_1-C_5 alkyl, halogen or NH_2 ,
 R^4 and R^5 which are the same or different are selected
 from H, $NH-Y'$ or NO_n wherein Y' is H, ribosyl, deoxyribosyl,
 arabinosyl, $R''XCHR'''$ wherein X is O or S and R'' is alkyl
 and R''' is H or alkyl, or substituted derivatives thereof,
 10 $n = 1$ or 2 ,
 or R^4 and R^5 together with the pyrimidine ring form a 5-or
 6-membered ring structure containing one or more hetero atoms,
 and pharmaceutically acceptable salts thereof,

- 15 with the proviso that R^2 is not NH^2 if R^4 and R^5 form
 a ring structure IX



20

wherein Y is H, ribosyl, deoxyribosyl, or $R''XCHR'''$ wherein X
 is O or S, R'' and R''' are alkyl, or substituted derivatives
 thereof,

25

and with the proviso that R is not phenyl in the following
 circumstances a) to h):

- a) if R^2 and R^5 are NH_2 and R^4 is NO or NO_2

30

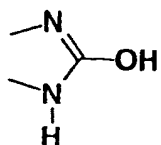
- b) if R^2 is NH_2 and R^4 and R^5 form a ring
 structure X



35

- c) if R^2 is NH_2 and R^4 and R^5 form a ring
 structure XI

- 8 -



XI

5

d) if R^2 is NH_2 , and R^4 is NO_2
and R^5 is H or CH_3

10 e) if R^2 , R^4 and R^5 are NH_2 ,

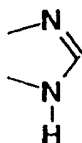
f) if R^2 and R^5 are NH_2 and R^4 is H,

g) if R^2 is H, and R^4 is NO_2 and R^5 is NH_2 , or

15

h) if R^2 is F or OH, and R^4 and R^5 form a ring structure

XII

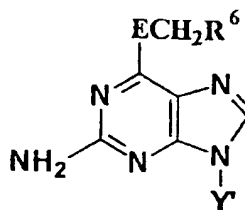


XII

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25 Certain Q^6 -substituted guanine derivatives within the scope of the general formula in WO 94/29312 but not published therein have been found to have a surprisingly advantageous combination of properties which justifies the selection of such derivatives from among the class defined in WO 94/29312.

30 In another aspect, the present invention provides guanine derivatives of formula XIII:



XIII

35

- 9 -

wherein

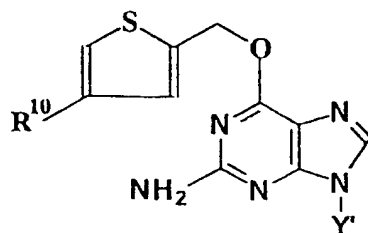
E is O or S,

Y' is as defined for formula II above,

- 5 R⁶ is a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N or S, or a substituted derivative thereof,
- 10 and pharmaceutically acceptable salts thereof, with the proviso that compounds published in WO 94/29312 are disclaimed.

In particular, the present invention selects advantageous compounds of formula XIV:

15



XIV

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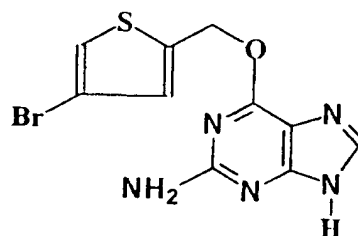
wherein

R¹⁰ is bromo, chloro or cyano, and

Y' is as defined for formula II.

- 25 Most preferably, R¹⁰ is bromo. A particularly preferred and selected compound is 2⁶-(4-bromothienyl)guanine having the formula XV:

30



XV

- This compound has an advantageous combination of properties
- 35 including potential for oral administration.

- 10 -

R or R⁶ may suitably be a 5- or 6-membered heterocyclic ring or a benzo derivative thereof, in which latter case the pyrimidine moiety may be attached to R or R⁶ at either the heterocyclic or
5 the benzene ring.

In preferred embodiments, R or R⁶ is a 5-membered ring containing S or O, with or without a second ring fused thereto.

10 Preferably, R or R⁶ is a heterocyclic ring having at least one S atom; more preferably, R or R⁶ is a 5-membered heterocyclic ring having at least one S atom; and most preferably, R or R⁶ is a thiophene ring or a substituted derivative thereof. Alternatively, R or R⁶ may be a heterocyclic ring having at least one O atom,
15 particularly, a 5-membered heterocyclic ring having at least one O atom and more particularly R or R⁶ may be a furan ring or a substituted derivative thereof. As another alternative, R or R⁶ may be a heterocyclic ring having at least one N atom, particularly R or R⁶ may be a 6-membered heterocyclic ring having at least one
20 N atom and in particular, R or R⁶ may be a pyridine ring.

The carbocyclic or heterocyclic ring fused to the heterocyclic ring in R or R⁶ may itself be bicyclic e.g. naphthalene.

25 In general the term "substituted derivative" as used in relation to any of the compounds of the invention means any substituted derivative whose presence in the compound is consistent with the compound having ATase depleting activity.

30 In the definition of Y or Y', the term "substituted derivative" includes further substitution by one or more of the following groups: hydroxy, halo, alkoxy, amino, alkylamino, amido or ureido. In a particularly preferred group of compounds, R" is hydroxy-substituted alkyl and R''' is H, so that Y' is
35 hydroxyalkoxymethyl, preferably having 1 to 10 carbon atoms in the alkoxy group.

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In the definition of R or R⁶, the term "substituted derivative" includes substitution of the heterocyclic ring(s) and/or carbocyclic ring(s) by one or more of the following groups: alkyl, alkenyl, alkynyl, alkoxy, aryl, halo, haloalkyl, nitro, cyano, azido, hydroxyalkyl, SO_nR⁷ where R⁷ is alkyl and n = 0, 1 or 2, or a carboxyl or ester group of the formula -COOR⁸ wherein R⁸ is H or alkyl. Halo, haloalkyl, cyano, alkylenedioxy, SO_nR⁷ (as defined above) and -COOR⁸ wherein R⁸ is alkyl are preferred substituents.

An alkyl, alkoxy, alkenyl, or alkynyl group preferably contains from 1 to 20, more preferably from 1 to 10 and most preferably from 1 to 5 carbon atoms. Halo includes iodo, bromo, chloro or fluoro. An aryl group preferably contains from 1 to 20, more preferably from 1 to 10 carbon atoms, particularly 5 or 6 carbon atoms.

One embodiment of the invention provides a pharmaceutical composition containing compounds of formula II or formula XIII, as defined above, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. Optionally the composition may also contain an alkylating agent such as a chloroethylating or methylating agent.

In a further embodiment, the present invention provides a method for depleting ATase activity in a host comprising administering to the host an effective amount of a composition containing a compound of formula II or formula XIII as defined above, or a pharmaceutically acceptable salt thereof, more particularly a pharmaceutical composition as defined above. This method may alternatively be defined as a method of depleting ATase mediated DNA repair activity in a host.

The invention further provides a method for treating tumour cells in a host comprising administering to the host an effective amount of a composition containing a compound of formula II or

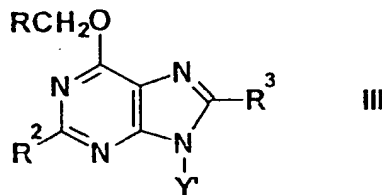
formula XIII as defined above or a pharmaceutically acceptable salt thereof, more particularly a pharmaceutical composition as defined above and administering to the host an effective amount of a composition containing an alkylating agent. The method may be used for treatment of neoplasms including those which are known to be sensitive to the action of alkylating agents e.g. melanoma and glioma and others whose resistance to treatment with alkylating agents alone may be overcome by the use of an inactivator according to the invention.

The term "pharmaceutically acceptable salts" as used in this description and the claims means salts of the kind known in the pharmaceutical industry including salts with inorganic acids such as sulfuric, hydrobromic, nitric, phosphoric or hydrochloric acid and salts with organic acids such as acetic, citric, maleic, fumaric, benzoic, succinic, tartaric, propionic, hexanoic, heptanoic, cyclopentanepropionic, glycolic, pyruvic, lactic, malonic, malic, o-(4-hydroxy-benzoyl)benzoic, cinnamic, mandelic, methanesulfonic, ethanesulfonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulfonic, p-chlorobenzenesulfonic 2-naphthalenesulfonic, p-toluenesulfonic, camphorsulfonic, 4-methyl-bicyclo[2.2.2]oct-2-ene-1-carboxylic, glucoheptonic, 4,4'-methylenebis(3-hydroxy-2-naphthoic), 3-phenylpropionic, trimethyl-acetic, tertiary butylacetic, lauryl sulfuric, gluconic, glutamic, hydroxynaphthoic, salicylic, stearic, or muconic, and the like.

Subject to the provisos above the preferred compounds of the
30 invention are those of:

Type 1

Formula III



- 13 -

wherein:

R is as defined for formula II, particularly furyl or thienyl
unsubstituted or substituted, preferably with a halogen such
as chlorine, bromine or fluorine, or with cyano

Y' is as defined for formula XIII, preferably Y' is H or

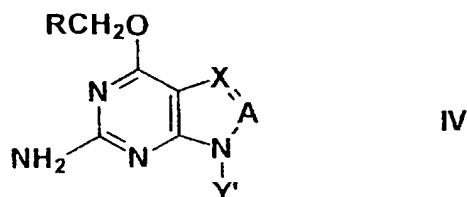
HOCH₂CH₂OCH₂-;

R² is H, NH₂, C₁-C₅ alkyl, preferably methyl, or
halogen, preferably fluorine;

R³ is H or OH;

Type 2

Formula IV



wherein:

R is as defined for formula II, particularly phenyl, thienyl
or furyl unsubstituted or substituted preferably with a
halogen such as chlorine, bromine or fluorine, or with cyano,
or phenyl having a methylenedioxy ring structure fused thereto;

Y' is as defined for formula XIII;

X is CH or N;

A is CH or N; and preferably when X = N, A = CH

Formula V

wherein:

R is as defined for formula II

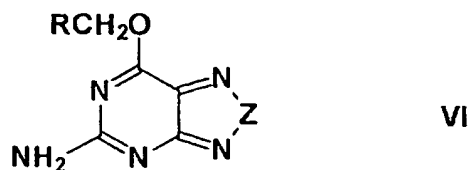
X is CH or N

A is CH or N;

Type 3

Formula VI

5



wherein:

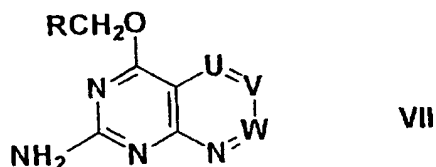
10 R is as defined for formula II, particularly, thienyl or furyl unsubstituted or substituted preferably with a halogen such as chlorine or bromine;

Z is O or S or CH = CH;

15 A particularly preferred group of compounds of this type are
 \underline{Q}^6 -(4-halothienyl)-8-thiaguanines, particularly
 \underline{Q}^6 -(4-bromothienyl)-8-thiaguanine.

Formula VII

20



wherein:

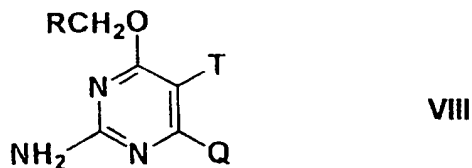
25 R is as defined for formula II;
 U is CH or N;
 V is CH or N;
 W is CH or N;
 provided that U, V and W are not all CH.

30

Type 4

Formula VIII

35



- 15 -

wherein:

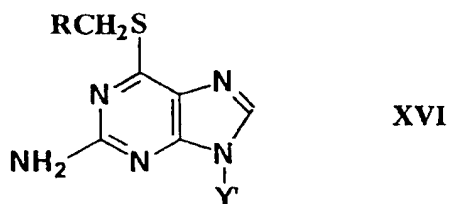
R is as defined for formula II, particularly thenyl or furyl optionally substituted with halogen preferably one or more of chlorine, bromine or fluorine;

T is H, NH_2 or NO_n where $n = 1$ or 2 ;

Q is H, NH_2 or NO_n where $n = 1$ or 2 ;

10 Type 5

Formula XVI



wherein

20 R is as defined for formula XIII

Y' is as defined for formula II

Brief Description of Drawings

25

The invention will be described in greater detail with reference to the accompanying drawings, in which:

30 Figures 1 to 4 are graphs showing the effect of pretreatment with compound B.4316 on Raji cell sensitization to different chemotherapeutic agents. Each graph plots percentage growth against the concentration ($\mu\text{g/ml}$) of the chemotherapeutic agent in the presence and absence of B.4316.

35 Figure 1 shows the effect of $1\mu\text{M}$ B.4316 pretreatment on Raji cell sensitization to temozolomide.

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Figure 2 shows the effect of 10 μ M B.4316 pretreatment on Raji cell sensitization to BCNU.

5 Figure 3 shows the effect of 10 μ M B.4316 pretreatment on Raji cell sensitization to fotemustine.

Figure 4 shows the effect of 10 μ M B4316 pretreatment on Raji cell sensitization to melphalan and cisplatin.

10

Figure 5 is a histogram showing the effect of 10 μ M B4316 pretreatment on Raji cell sensitization to different chemotherapeutic agents, measured as sensitization factor (SF, defined below) based on D₅₀ except for fotemustine where SF is
15 based on D₈₀.

Figure 6 is a similar histogram showing the effect of 10 μ M B4349 pretreatment on Raji cell sensitization to different chemotherapeutic agents, with SF as for Figure 5.

20

Figure 7 is a series of histograms showing the inactivation of ATase in A375M tumours and murine host tissues two hours after interperitoneal (i.p.) administration of various inactivator compounds at 5mg/kg. Inactivation was calculated as % of control
25 ATase activity, measured as fm/mg protein.

Figure 8 is a graph showing the kinetics of ATase depletion and recovery in A375M tumours and murine host tissues after administration of B.4363 (20 mg/kg i.p.). The graph plots % of
30 control ATase activity against time (hours).

Figure 9 is a graph of percentage residual activity of pure recombinant human ATase following incubation with increasing concentrations of inactivators Q⁶-benzylguanine (BeG),
35 Q⁶-thenylguanine (B.4205) and Q⁶-(4-bromothienyl)guanine (B.4280). The line at 50% residual activity is used for calulating I₅₀ values i.e. the concentration of inactivator required to

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produce a 50% reduction in ATase activity. The I_{50} values shown are extrapolated from the curves. Preincubation was for 1 hour after which [^3H]-methylated substrate was added to determine
5 residual activity of ATase.

Figure 10A is three graphs of percentage cell growth against temozolomide concentration ($\mu\text{g/ml}$) showing the effect of pretreatment with BeG, B.4205 and B.4280 ($0.5\mu\text{M}$ final concentration)
10 on the sensitivity of Raji cells to the growth inhibitory effects of temozolomide. Inactivator or vehicle was given 2 hours prior to temozolomide.

Figure 10B is a histogram for the inactivators of Figure 10A
15 showing the sensitization factor based on D_{50} of Raji cells to growth inhibition by temozolomide.

Figure 11 is a histogram of ATase activity (fm/mg) against time (hours) showing the effect of ATase inactivators BeG, B.4205
20 and B.4280 on ATase activity in human melanoma xenografts grown in nude mice. Animals were given a single dose of the inactivators intraperitoneally (i.p.) at 30mg/kg or 60mg/kg and sacrificed after the times shown.

Figure 12 is a histogram showing the effect of ATase
25 inactivators on ATase activity (fm/mg) in human melanoma xenografts grown in nude mice. Animals were given B.4205 or temozolomide alone or B.4205 or B.4280 in combination with temozolomide (50mg/kg) i.p. at the doses shown on three consecutive days (except where
30 indicated) and sacrificed 24 hours after the final dose. The vehicles were corn oil for the inactivators and PBS (20%DMSO) for temozolomide.

Figure 13 is a histogram showing the effect of ATase
35 inactivators on ATase activity in livers of nude mice. Animals were given the B.4205 or temozolomide alone or B.4205 or B.4280 in combination with temozolomide (50mg/kg, i.p.) at the doses shown on

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three consecutive days (except where indicated) and sacrificed 24 hours after the final dose.

5 Figure 14A is a graph of % tumour growth against time (days) showing the effect of B.4205 on the sensitivity of human melanoma xenografts to growth inhibition by temozolomide. Animals were untreated, given temozolomide alone (100mg/kg, i.p.) or B.4205 (5, 10 or 20mg/kg i.p.) followed 1 hour later by temozolomide (100mg/kg, 10 i.p.) on five consecutive days. Tumour growth was monitored as described. The data from a number of separate studies are presented.

Figure 14B is a graph of number of surviving mice against time (days) showing survival of animals (tumour-bearing nude mice) used 15 in the study shown in Figure 14A. Groups of animals in which the xenografts had reached the maximum size were terminated.

Figure 15A is a graph showing the effect of B.4280 on the sensitivity of human melanoma xenografts to growth inhibition by 20 temozolomide. Animals were untreated, given temozolomide alone (100mg/kg, i.p.) or B.4280 alone (20mg/kg, i.p.) or B.4280 (1, 5, 10 or 20 mg/kg, i.p.) followed 1 hour later by temozolomide (100mg/kg, i.p.) on five consecutive days. Tumour growth was monitored as described. The data from a number of separate studies are presented.

25 Figure 15B is a graph showing the survival of the animals (tumour-bearing nude mice) used in the study shown in Figure 15A. Groups of animals in which the xenografts had reached the maximum size were terminated.

30 Figure 16A is a graph of % tumour growth against time (days) showing the comparison of the effect of B.4280 given i.p. and orally (p.o.) on the sensitivity of human melanoma xenografts to growth inhibition by temozolomide. Animals were untreated, given 35 temozolomide alone (100mg/kg) or B.4280 alone (20mg/kg, i.p.) or B.4280 (20mg/kg, i.p.) or B.4280 (30mg/kg, p.o.) followed 1 hour later by temozolomide (100mg/kg, i.p.) on five consecutive days.

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Tumour growth was monitored as described. The data from a number of separate studies are presented.

5 Figure 16B is a graph showing the survival of the animals used in the study shown in Figure 16A. Groups of animals in which the xenografts had reached the maximum size were terminated.

10 Figure 17 is a graph showing the survival of animals in a comparative test of the effects of BeG, B.4205 and B.4280 in combination with temozolomide (TZ) in non-tumour-bearing DBA₂ mice. Animals were given temozolomide alone (100mg/kg i.p) or BeG (10 or 20mg/kg i.p.), B.4205 (10 or 20 mg/kg i.p.) or B.4280 (10 or 20 mg/kg i.p.) followed one hour later by temozolomide (100mg/kg
15 i.p.) on five consecutive days.

20 Figures 18 to 21 consist of pairs of graphs showing the kinetics of ATase depletion and recovery in various tumours and murine host tissues after administration of B.4280 at the doses indicated. The graphs plot ATase activity (fm/mg protein) and % of control ATase activity against time (hours):

25 Figure 18 relates to B.4280 (20 mg/kg i.p.) in A375M tumours and other tissues.

 Figure 19 relates to B.4280 (30mg/kg p.o.) in A375M tumours and other tissues

30 Figure 20 relates to B.4280 (30mg/kg i.p.) in MCF-7 tumours and other tissues.

 Figure 21 relates to B.4280 (20mg/kg i.p.) in DU-145 tumours and other tissues.

35 Figure 22 is a graph of % tumour growth against time (days) showing the effect of B.4280 on the sensitivity of MCF-7 tumours to growth inhibition by temozolomide. Animals were untreated, were

- 20 -

given temozolomide alone (100 mg/kg, i.p.) or B.4280 (PaTrin-2) (20mg/kg i.p.) alone, or B.4280 (20mg/kg i.p.) followed 1 hour later by temozolomide (100mg/kg i.p.) on five consecutive days.

5

Figure 23 consists of graphs of % tumour growth, number of surviving mice and mean weight (g) against time (days) showing the effect of a single dose of B.4280 (PaTrin-2) on the sensitivity of melanoma tumours to growth inhibition by a single dose of fotemustine. Animals were given fotemustine (20mg/kg i.p.) alone, or B.4280 (30mg/kg p.o) followed 1 hour later by fotemustine (20mg/kg i.p.).

Figure 24 consists of graphs of % tumour growth and number of surviving mice against time (days) for sensitization of A375M tumours with B.4205 (PaTrin-1) and B.4280 20mg/kg pretreatment followed by 150mg/kg temozolomide using a 5 day schedule as for Figure 22.

Figure 25 consists of graphs of % tumour growth, number of surviving mice and mean weight (g) against time showing sensitization of A375M tumours to temozolomide (100mg/kg i.p.) following administration of 20mg/kg B.4349 or B.4351 (i.p.).

Figure 26 is a figure showing ATase activities in A375M tumours and murine host tissues at 2 hours and 24 hours following i.p. administration of 90mg/kg B.4335.

In the specification the abbreviations "1h" or "2h" etc. mean "1 hour", "2 hours" etc.. In the drawings the abbreviations "Temo" and "Tz" refer to temozolomide.

Figure 27 consists of graphs of % tumour growth and weight (% of day 1 value) against time (days) showing tumour DU-145 prostate xenograft growth after temozolomide (100mg/kg/day) and/or B.4280 (PaTrin-2) (20mg/kg/day) days 1-5. Points are the means of values from at least 4 mice. Growth delays in each group were (p value):

- 21 -

PaTrin-2 alone 0.1 days (>.05); temozolomide alone 7.8 (>.05).
Both agents 15.3 (0238).

5 Figure 28 is a reaction scheme for synthesis of
 $\text{Q}^6\text{[}^3\text{H]-(4-bromophenyl)guanine}$.

 Figure 29 shows co-chromatography of authentic B.4280 and
 radioactivity in the product of $\text{Q}^6\text{[}^3\text{H]-(4-bromophenyl)guanine}$
10 synthesis. Shading indicates counts recovered (LH axis) and the
 line OD at 254nm (RH axis).

 Figure 30 shows transfer of radioactivity from
 $\text{Q}^6\text{[}^3\text{H]-(4-bromophenyl)guanine}$ to rhATase after one hour
15 incubation at 37°C.

Description of the Preferred Embodiments

20 Examples of compounds of the invention are shown in Tables 1a
 and 1b. They were synthesized by the procedures presented below,
 adapted as appropriate.

Type 1

- 25 A. Q^6 -Substituted hypoxanthines were made by the action of
 alkoxide RCH_2ONa on the quaternary salt
 $\text{N,N,N-trimethyl-1H-purin-6-aminium chloride}$.¹
- B. Q^6 -Substituted 2-methylhypoxanthines were made similarly,
30 from the quaternary salt from diazabicyclooctane (DABCO) and
 6-chloro-2-methylpurine.²
- C. Q^6 -Substituted 2-fluorohypoxanthines were made by
 diazotisation of the corresponding guanines using sodium
35 nitrite and concentrated fluoboric acid at -25°C.³

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D. \underline{Q}^6 -Substituted 9-(2-hydroxyethoxymethyl)guanines were made by condensing the corresponding guanines after silylation with 2-acetoxyethoxymethyl bromide in the presence of mercuric cyanide followed by saponification of the \underline{Q} -acetyl group.⁴

E. \underline{Q}^6 -Substituted 8-hydroxyguanines were made from 6-hetarylmethyl-2,4,5-triaminopyrimidines and 1, 1-carbonyldiimidazole in DMF.⁵ Reaction of 6-chloro-2,4-diaminopyrimidine with alkoxide in DMSO, followed by nitrosation with sodium nitrite in aqueous acetic acid and reduction using sodium hydrosulphite in aqueous DMF, gave the 2, 4, 5-triamines.

15 Type 2

A. \underline{Q}^6 -Substituted 8-azaguanines were made from the above triamines and sodium nitrite in aqueous acetic acid.⁶

20 B. \underline{Q}^6 -Substituted 8-aza-7-deazaguanines were made from the alkoxide RCH_2ONa and 2-amino-6-chloro-8-aza-7-deazapurine⁷ in sulfolane or from the DABCO quaternary salt (in DMSO solvent) derived from it.

25 Type 3

A. \underline{Q}^6 -Substituted 8-oxaguanines were made by lead tetraacetate oxidation⁸ of 6-hetarylmethyl-2,4-diamino-5-nitrosopyrimidines obtained as under Type IE.

B. \underline{Q}^6 -Substituted 8-thiaguanines were made from the triamine intermediates under Type IE and \underline{N} -tosylthionylimine in pyridine.⁹

35 C. \underline{Q}^4 -Substituted pterins were made from these triamines and glyoxal with sodium metabisulphite.¹⁰

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Type 4

A and B.

5 These pyrimidines were obtained as under Type IE.

C. O^6 -Substituted 2,4-diamino-5-nitropyrimidines were made by
the action of alkoxide RCH_2ONa in DMSO on
6-chloro-2,4-diamino
10 -5-nitropyrimidine.¹¹

Type 5

S^6 -Substituted 6-thioguanines were prepared from the
15 thiolate RCH_2SNa and the quaternary salt 2-amino-N,N,N-trimethyl-1
H-purin-6-aminium chloride (WO 94/29312).

O^6 -Substituted guanines as listed in Tables 6a and 6b were
made by the standard preparation as described in WO 94/29312,
20 usually with 3mmol alcohol RCH_2OH per mmol quaternary salt.

The alcohols were made as described in WO 94/29312 by sodium
borohydride reduction of the corresponding aldehydes, with two
exceptions. For 4-bromophenyl alcohol¹² required for B.4280 the
25 aldehyde is commercially available. 5-Chlorothiophen-2-aldehyde¹³
and 5-methylthiothiophen-2-aldehyde¹⁴ were prepared by Vilsmeier
reaction on 2-chlorothiophen and 2-methylthiothiophen respectively.
Sodium borohydride reduction of the methylthioaldehyde followed by
sodium periodate oxidation¹⁵ of the resulting methylthioalcohol
30 yielded the methylsulphinyalcohol required for B.4294. Reduction
of the chloroaldehyde gave 5-chlorophenyl alcohol¹⁶ for B.4281.

Several other aldehydes were obtained by halogenation of the
appropriate thiophen aldehyde or furfural. Thus, direct bromination
35 gave 5-bromofurfural¹⁷ and thence the alcohol¹⁸ for B.4336.
Halogen in presence of aluminium chloride on thiophen-2-aldehyde
yielded 4-chlorothiophen-2-aldehyde¹⁹ (for the alcohol for

- 24 -

B.4298), on thiophen-3-aldehyde yielded
2-bromothiophen-4-aldehyde²⁰ (and eventually B.4313), and on
5-chlorothiophen-2-aldehyde yielded
5 4,5-dichlorothiophen-2-aldehyde²¹ (for the alcohol²² for B.4318).

Cyanoaldehydes were obtained from copper cyanide and the
corresponding bromoaldehydes in refluxing dimethylformamide.
5-Cyanothiophen-2-aldehyde²³ and its 4-cyano isomer²⁴ then gave
10 the 5-cyano and 4-cyano²⁵ alcohols, for B.4283 and B.4317
respectively.

4-Methoxythenyl alcohol²⁶ (for B.4300) was prepared as
described from 2,3-dibromosuccinic acid and methyl thioglycollate,
15 and ultimate reduction of the methyl ester (not aldehyde in this
case) by lithium aluminium hydride and 2-chloro-4-picolyl
alcohol²⁷ (for B.4321) by sodium borohydride reduction²⁸ of the
corresponding acid chloride, made in turn from reaction²⁹ of
phosphorus oxychloride/pentachloride on isonicotinic acid N-oxide.

20 For B.4282, 3-pyridinemethanol N-oxide is commercially
available. 5-Methylsulphonylthenyl alcohol (for B.4309) was
obtained by m-chloroperbenzoic acid (MCPBA) oxidation of the alcohol
resulting from reduction of 5-methylthio-2-thiophenecarboxaldehyde
25 30.

6-Chloro-3-pyridinemethanol (for B.4319) and
5-bromo-3-pyridinemethanol (for B.4320) were made by treatment of
6-chloro and 5-bromonicotinic acids respectively with phosphorus
30 oxychloride/pentachloride and reduction of the resulting acid
chlorides with sodium borohydride²⁸. Isothiazole-4-methanol (for
B.4354) was obtained by reduction of the corresponding methyl ester
(A. Adams and R. Slack, J. Chem. Soc. 1959, 3061) with lithium
aluminium hydride (M. Hatanaka and T. Ishimaru, J. Med. Chem. 16,
35 1973, 978).

4-bromo-2-thiophenecarboxaldehyde was converted into the

- 25 -

4-lithio derivative (A.L. Johnson, J. Org. Chem., 41, 1976, 1320) of its ethylene acetal and reaction of this organometallic with dimethyl disulphide followed by acid hydrolysis gave

5 4-methylthio-2-thiophenecarboxaldehyde (R. Noto, L. Lamartina, C. Arnone and D. Spinelli, J. Chem. Soc., Perkin Trans., 2, 1987, 689). Sodium borohydride reduced this aldehyde to the 4-methylthio alcohol (for B.4356), which in turn with one of two equivalents of MCPBA yielded the 4-methylsulphinyl and 4-methylsulphonyl alcohols (for

10 B.4377 and B.4361 respectively). Reaction of the above organometallic with naphthalene-2-sulphonyl azide (A.B. Khare and C.E. McKenna, Synthesis, 1991, 405) and sodium pyrophosphate followed by hydrolysis by the method (P. Spagnolo and P. Zanirato, J. Org. Chem., 43, 1978, 3539) for the preparation of other

15 azidothiophene aldehydes gave 4-azido-2-thiophenecarboxaldehyde leading to the alcohol for B.4373.

5-Iodo-3-thiophenemethanol (for B.4357) came from the aldehyde obtained by treatment of 3-thiophenecarboxaldehyde with iodine-iodic

20 acid-sulphuric acid (R. Guillard, P. Fournari and M. Person, Bull. Soc. Chim. France, 1967, 4121).

2-Naphtho[2, 1-b]thienylmethanol (for B.4366) was prepared by lithium aluminium hydride reduction of the corresponding carboxylic

25 acid (M.L. Tedjamulia, Y. Tominaga, R.N. Castle and M.L. Lee, J. Heterocycl. Chem., 20, 1983, 1143). 5-Phenylthienyl alcohol (m.p. 91.5°C, for B.4378) resulted from sodium borohydride reduction of the aldehyde (P. Demerseman, N.P. Buu-Hoi and R. Royer, J. Chem. Soc., 1954, 4193) obtained by Vilsmeier reaction of

30 2-phenylthiophene (from Gomberg-Bachmann reaction (N.P. Buu-Hoi and N. Hoan, Rec. trav. chim., 69, 1950, 1455) of benzenediazonium chloride and alkali with thiophene).

By way of specific example, the preparation of

35 Q^6 -(4-bromophenyl)guanine (B.4280) will now be described.

Preparation of $\underline{0}^6$ -(4-bromophenyl)guanine

A solution of 4-bromophenyl alcohol¹² [4.63g, 24mmol; R_f 0.38 in TLC (PhMe-MeOH, 4:1)] in DMSO (4ml) was treated cautiously with sodium hydride (60% in oil; 0.64g, 16mmol). After 1 hour's stirring, 2-amino-N,N,N-trimethyl-1H-purin-6-aminium chloride (1.83g, 8mmol) was added. After 1 hour's further stirring, acetic acid (1.3ml) followed by ether (240ml) was added and the solid filtered off after 1-2h. Removal of solvents and excess of alcohol (b.p. 85-90°C/0.4mm) from the filtrate yielded a negligible second fraction (17mg). The main crop was triturated with water (10ml), affording substantially pure product (1.89g, 73%) with R_f 0.22 in TLC (PhMe-MeOH, 4:1). It was recrystallized by dissolving in hot methanol (100ml) and then concentrating. Analytical data are given in Tables 6a and 6b, together with data for other compounds. Other typical synthetic procedures are described by way of example in a special section later in this text.

Compounds of formula II or XIII in which Y' is R''XCHR''' and R''' is alkyl (seco-nucleosides) may be prepared by an analogous preparation to the reaction of $\underline{0}^6$ -benzylguanine with -chloro-ethers (MacCoss et al., Tetrahedron Lett.; European Patent Application No. 184,473., loc. cit.) or with alkyl bromides (e.g. Kjellberg, Liljenberg and Johansson, Tetrahedron Lett., 1986, 27, 877; Moschel, McDougall, Dolan, Stine, and Pegg, J. Med. Chem., 1992, 35, 4486).

Typical "sugar" components corresponding to R''XCHR''', leading to seco-nucleosides, are made by methods described in e.g. McCormick and McElhinney, J. Chem. Soc., Perkin Trans. 1, 1985, 93; Lucey, McCormick and McElhinney, J. Chem. Soc., Perkin Trans. 1, 1990, 795.

Compounds of formula II or XIII in which Y is ribosyl or deoxyribosyl (nucleosides) may be prepared by methods analogous to the syntheses of $\underline{0}^6$ -benzylguanine riboside and 2-deoxyriboside (Moschel et al. 1992; cf. Gao, Fathi, Gaffney et al., J. Org. Chem.,

1992, 57, 6954; Moschel, Hudgins and Dipple, J. Amer. Chem. Soc., 1981, 103, 5489) (see preparation of Ribosides above).

5 Industrial Applicability

The amount of the compound of the present invention to be used varies according to the effective amount required for treating tumour cells. A suitable dosage is that which will result in a concentration of the compound of the invention in the tumor cells to be treated which results in the depletion of ATase activity, e.g. about 1 - 2000 mg/kg body weight, and preferably 1 - 800 mg/kg body weight, particularly 1-120 mg/kg body weight, prior to chemotherapy with an appropriate alkylating agent.

15

The pharmaceutical composition of the invention may be formulated in conventional forms with conventional excipients, as described for example in WO 91/13898 and WO 96/04281 and U.S. Patents 5,091,430 and 5,352,669, the contents of which are incorporated herein by reference in their entirety. The composition may contain the inactivator according to the invention together with an appropriate alkylating agent; or the composition may comprise two parts, one containing the inactivator and the other containing the alkylating agent. The method of administering the compounds of the invention to a host may also be a conventional method, as described in WO 91/13898 for example. For administration of an inactivator according to the invention to patients, the pharmaceutical composition may suitably contain the inactivator in a suitable vehicle such as 40% polyethyleneglycol 400 in saline solution, or in saline or 3% ethanol (in saline), for intravenous injection, or in a powder form in suitable capsules for oral administration.

Alkylating agents may be administered in accordance with known techniques and in conventional forms of administration, as described in WO 91/13898 for example or preferably as a single dose immediately after or up to 24 hours after but preferably around 2

- 28 -

hours after administration of the ATase inactivating agents and also at doses lower than those used in standard treatment regimen. A reduction in dose may be necessary because the inactivators would generally be anticipated to increase the toxicity of the alkylating agents. Examples of chloroethylating agents include 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), fotemustine, mitozolomide and clomesone and those described in McCormick, McElhinney, McMurphy and Maxwell J. Chem. Soc. Perkin Trans. I, 1991, 877 and Bibby, Double, McCormick, McElhinney, Radacic, Pratesi and Dumont Anti-Cancer Drug Design, 1993, 8, 115. Examples of methylating agents include temozolomide (British Patent GB 2 104, 522 and U.S. Patent 5,260,291 the contents of which are incorporated herein in their entirety) and dacarbazine, procarbazine, and streptozocin.

Methods

20 6 -alkylguanine-DNA-alkyltransferase assay

Varying amounts of recombinant ATase or cell/tissue extracts were incubated with [3 H]-methylnitrosourea-methylated calf thymus DNA (specific activity, 17Ci/mmol) at 37°C for 1 hour in a total volume of 300 μ l buffer I/[50mM Tris/HCl (pH8.3), 3mM dithiothreitol (DTT), 1mM EDTA] containing 1mg/ml bovine serum albumin (IBSA) for recombinant ATases and tissue extracts, or 1.1ml buffer I for cell extracts. After incubation, bovine serum albumin (100 μ l of 10mg/ml in buffer I) and perchloric acid (100 μ l of 4M perchloric acid for 30 300 μ l volumes and 400 μ l for 1.1ml volumes) and 2ml of 1M perchloric acid were added. Samples were then heated at 75°C for 50 minutes to hydrolyze the DNA. Samples were then centrifuged at 3,000rpm for 10 minutes and the precipitate washed once with 4ml of 1M perchloric acid, before being resuspended in 300 μ l of 0.01M sodium hydroxide and dissolved in 3ml of aqueous scintillation fluid (Ecoscint A, 35 National Diagnostics). Counting efficiency was approximately 30%. ATase specific activity was calculated from the region where the

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activity was proportional to the amount of extract added, since with higher amounts of extracts the reaction becomes substrate limiting. ATase activity is expressed as fmol methyl transferred to protein per mg of total protein in the extract.

Method of Purification of Recombinant ATases

The cDNA cloning and overexpression of the human ATase has been reported previously³⁰. Purification of the recombinant proteins was achieved either by affinity chromatography through a DNA-cellulose column as described by Wilkinson *et al.*,^{31, 32} or by DEAE-cellulose ion-exchange chromatography. For the latter, the ATase protein was partially purified by ammonium sulphate precipitation (30 - 60%) and dialyzed against 10 mM Tris-HCl pH 7.5, 1 mM DTT, 2 mM EDTA, 10% glycerol, before loading on a DEAE-cellulose column. The ATase was then eluted with a 0-0.1 M NaCl gradient. The purified human ATase protein retained activity for more than one year when stored at high concentration at -20°C in buffer I [50 mM-Tris/HCl (pH 8.3)/3 mM-dithiothreitol/1 mM-EDTA] and could be thawed and refrozen several times without substantial loss of activity.

Incubation with Inactivators and ATase assay

25

Compounds to be tested were dissolved in DMSO to a final concentration of 10 mM and diluted just before use in buffer I. Recombinant ATase was diluted in buffer I containing 1 mg/ml bovine serum albumin (IBSA) and titrated as described above in order that the reaction be conducted under ATase, and not substrate, limiting conditions. In each assay, fixed amounts of ATase (60-75 fmol) were incubated with varying amounts of O⁶-benzylguanine, or test compound in a total volume of 200 µl of IBSA containing 10 µg of calf thymus DNA at 37°C for 1 hour. The [³H]-methylated-DNA substrate (100 µl containing 4 µg of DNA and 100 fmol of O⁶-methylguanine) was then added and incubation continued at 37°C for 1 hour, until the reaction was complete. Following acid

- 30 -

hydrolysis of the DNA as described above the [^3H]-methylated protein was recovered and quantitated by liquid scintillation counting. I_{50} is the concentration of inactivator required to
5 produce a 50% reduction in ATase activity under the above conditions.

Cell Culture and preparation of extracts

Mammalian cells including Raji cells (a human lymphoblastoid cell line from a Burkitt's lymphoma), A375M cells (human melanoma cells),
10 MCF-7 cells (human breast cancer cells) and PC3 and DU145 (both human prostate cancer cells) were cultured under standard conditions. For example, Raji cells were grown in suspension culture in RPMI medium supplemented with 10% horse serum. Cell pellets were resuspended in cold (4°C) buffer I containing $2\mu\text{g/ml}$ leupeptin
15 and sonicated for 10 seconds at $12\mu\text{m}$ peak to peak distance. After cooling in ice, the cells were sonicated for a further 10 seconds at $18\mu\text{m}$. Immediately after sonication, $10\mu\text{l/ml}$ of phenylmethanesulphonylfluoride (PMSF 8.7 mg/ml in 100% ethanol) was added and the sonicates centrifuged at 15 000cpm for 10 minutes at
20 4°C to pellet cell debris. The supernatant was transferred to a tube on ice and kept for determination of ATase activity (see above).

Stability of Inactivators at 37°C by Spectrophotometry.

Inactivators (10mM in DMSO) were diluted to 0.1mM in prewarmed
25 degassed PBS (pH 7-7.2). PBS (Phosphate buffered saline) is 0.8% NaCl, 0.02% KCl, 0.15% $\text{Na}_2\text{H}_2\text{PO}_4$, 0.02% KH_2PO_4 . Samples were immediately transferred to a CARY13 spectrophotometer (cuvette block held at 37°C) and scanned at an appropriate wavelength (according to the spectral properties of the compound) at 5-10
30 minute intervals for up to 80 hours. The results were expressed as percentage absorbance change versus time and $T_{1/2}$ values (half life) extrapolated from this. In the tables the results of these tests are identified by "in PBS" or "by Spec".

35 Stability of Inactivators by ATase Assay

Inactivators ($10\mu\text{M}$ in DMSO) were diluted to the appropriate concentration (I_{90} calculated from previous I_{50} determination

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data) in buffer I without DTT and incubated for varying times at 37°C. Samples were then taken for use in the competition assay to assess the compound's ability to inactivate human ATase. The results were expressed as reduction in inactivating activity versus time and T 1/2 values extrapolated from this.

Inactivation of ATase activity in Raji cells.

Raji cells were diluted to between 5×10^5 /ml and 10^6 /ml in medium containing either the appropriate concentration of inactivator or an equivalent volume of vehicle (DMSO). Following incubation at 37°C for 2 hours the cells were harvested by centrifugation, washed twice with PBS and the resulting cell pellets (between 5×10^6 and 10^7 cells per pellet) stored at -20°C. ATase activity was determined as described above, in duplicate cell extracts and expressed as the percentage activity remaining, based on that present in the untreated controls (350-450 fm/mg depending on the assay). I_{50} (i.e concentration of inactivator required to reduce ATase activity by 50%) values were extrapolated from this data.

Sensitization of Mammalian cells to Cytotoxic Agents.

Sensitization of mammalian cells to the cytotoxic effects of BCNU, temozolomide and other cytotoxic agents following a 2 hour pretreatment with inactivator was analysed using an XTT-based growth inhibition assay²². Cells were plated in 96 well plates (for example in the case of Raji cells at 500 cells/well) and incubated at 37°C for 30 minutes prior to the addition of medium containing either the appropriate concentration of inactivator or an equivalent volume of vehicle. Following a 2 hour incubation at 37°C, medium containing either increasing doses of cytotoxic agent or equivalent vehicle was added and the cells allowed to grow for 6 days. At this time XTT solution was added and the cells incubated for a further 4 hours at 37°C. The resulting red/orange formazan reaction product was quantified by measuring absorption at 450nm on a microtitre platereader.

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From this data the percentage growth of cells relative to that in control wells was determined for a range of BCNU, temozolomide or other cytotoxic agent doses in both the presence and absence of inactivator. Sensitization factor (SF) based on D_{50} (D_{50}^C/D_{50}^I) was determined by dividing the D_{50} (i.e. dose at which there was 50% growth versus the controls untreated with alkylating agent) calculated for the cytotoxic agent alone (D_{50}^C) by that for the cytotoxic agent plus inactivator (D_{50}^I). A value of one (1) thus indicates no sensitization by the inactivator. Comparable Sensitization factors were also determined in some cases based on D_{60} and D_{80} , i.e. the dose at which there was respectively 60% or 80% growth compared to the untreated controls. In Table 3 the Sensitization Factor D_{50}^C/D_{50}^I is shown as D_{50} control / D_{50} 'B', with the letter 'B' referring to the inactivator compound.

Xenograft Studies

20 Animals

Swiss mouse derived athymic male mice (o/nu) weighing between 20-30g were obtained from ZENECA Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4T6, England. Animals were housed 4-5/cage in filter top cages and had access to food and water *ad libitum*. All animals were maintained under a controlled 12h-light-12h-dark cycle. These animals were used for all tests except those which are shown in Figures 11 and 17 and Table 8, as mentioned below.

30 Cells

A375M (human melanoma) and DU145 (human prostate cancer) cells were grown in DMEM containing 10% foetal bovine serum (FBS). MCF-7 (human breast cancer cells) were grown in DMEM containing 10% FBS supplemented with 100iu insulin.

35

Tumours

A375M, DU145 and MCF-7 cells (10^6) in 100 μ l PBS were

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injected subcutaneously into the right-hand flank of 8-10 week old o/nu athymic mice. These cells were allowed to develop into a tumour for 3-4 weeks (A375M and DU145 cells) and 4-6 weeks (MCF-7 cells). Once established, tumours were maintained by subcutaneous implantation of 2mm³ blocks into the right-hand flank of athymic o/nu mice. MCF-7 tumours are oestrogen positive and require oestrogen for growth. This was supplied as a subcutaneous implant (see below) at the tail base simultaneously to the tumour implant and monthly thereafter.

Preparation of Oestrogen Pellets

468mg β -oestradiol was added to 9.7g silastic and mixed until evenly distributed. 1.1g of curing agent was added and the whole mixture spread into 3 (26mm x 12mm x 1mm) glass fomers. These were then incubated at 42°C overnight before being cut into 2mm x 2mm x 1mm cubes, so that each pellet contained 2mg estradiol.

ATase Depletion Experiments

Tumours were implanted as previously described and left 3-6 weeks to establish depending on tumour type. An inactivator was homogenized in corn oil at 5mg/ml before administration by interperitoneal injection (i.p.) or oral gavage (p.o.). Mice were sacrificed at various times up to 72h and tumours and murine tissues taken for ATase assay. Samples were snap frozen and stored at -20°C until analysis.

Tumour Sensitization Experiments

O/nu mice were treated with the appropriate dose of the inactivator as indicated (4mg/ml in corn oil) or the appropriate vehicle as a control 1 hour prior to administration of the appropriate dose of the cytotoxic agent (e.g. temozolomide 6mg/ml in PBS + 20% DMSO) or fotemustine or BCNU (2mg/ml in PBS + 3% ethanol) using the doses and schedules indicated.

Tumour Measurements

Animals were weighed twice weekly and xenograft tumour

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measurements taken using digital calipers. Tumour volume was calculated using the formula $(h \times w \times l) \pi / 6$. Measurements continued until the tumour reached the maximum allowable volume (i.e. 1cm^3). Results were expressed as percentage tumour growth using day 1 tumour volumes as controls.

In the tests on the compounds shown in Table 6 and in Figures 9 to 17, the Methods used were as described in WO 94/29312. The following items a) to c) are also to be noted:

a) Standard ATase assay

ATase substrate DNA was prepared by incubation of purified calf thymus DNA with N-[^3H]-methyl-N-nitrosourea (18.7 Ci/mmol, Amersham International). Cell or tissue extracts were incubated with [^3H]-methylated-DNA substrate (100 μl containing 6.7 μg of DNA and 100fmol of O 6 -[^3H]methylguanine) at 37°C for 60 mins.. Following acid hydrolysis of the DNA as previously described³³ the [^3H]-methylated protein was recovered and quantitated by liquid scintillation counting.

b) Drug Treatment

Mice were treated with the inactivator as a suspension in corn oil by intraperitoneal injection (i.p.) or by oral gavage (p.o.) 60 mins prior to temozolomide (100mg/kg in 20%DMSO in phosphate-buffered saline) which was always given by intraperitoneal injection: this schedule was repeated on days 1 to 5 inclusive. Controls received vehicle alone, inactivator alone or temozolomide alone.

c) Animals

The mice in the tests shown in Figure 11 and Table 8 were BALB-C derived athymic male mice (nu/nu athymic) from the in-house breeding colony of the Paterson Institute for Cancer Research as described in WO 94/29312 (Animal Services Unit-ASU Mice).

- 35 -

The mice in the tests shown in Figures 12-16 were Swiss mouse derived athymic male mice (o/nu athymic) as described above.

5 The mice in the tests shown in Figure 17 were DBA₂ mice from the in-house breeding colony of the Paterson Institute for Cancer Research (Animal Services Unit), originally from the Jackson Laboratory in 1970.

10 **Test Results**

 The results of the ATase depletion assay on the compounds of Table 1 are shown in Table 2 or Table 3. Many of the compounds tested were more efficient in inactivating ATase than Q⁶-benzylguanine. In accordance with the results in WO 94/29312
15 the parent application, compounds in which R is a heterocyclic group were more efficient than the comparable compounds having benzyloxy side chains. In general the compounds in which RCH₂ is substituted or unsubstituted thenyl were the most efficient, the most preferred being halo- substituted thenyl having its halo
20 substituent in a 1,3-relationship with the methyleneoxy group attached to the pyrimidine residue.

 Tables 3, 4 and 5 summarize data for a number of parameters. Table 3 includes depletion assay results for recombinant ATase of
25 the following types:

 hAT = human
 mAT = mouse
 rAT = rat
30 chAT = Chinese hamster
 ogt = E. Coli ogt gene
 ada = E. Coli ada gene

 The combinations of properties for the various inactivators
35 can be seen in the tables. The following surprising points are noted in particular:

- 36 -

B.4316 is a compound of surprisingly high water solubility. B.4335 is a compound that is unexpectedly much more effective in the inactivation of ATase in Raji cells than of pure recombinant protein: generally, the I_{50} for inactivation of recombinant ATase *in vitro* is lower or similar to that in cultured cells.

B.4343 is a compound that has a very low I_{50} for ATase *in vitro* but is not as capable as agents with higher I_{50} s (e.g. B.4335) in the sensitization of Raji cells to the growth inhibitory effects of temozolomide. A similar example is B.4351 versus B.4349.

B.4316 was twice as effective as B.4280 but sensitization to temozolomide of Raji cells was almost identical. Thus different cell lines may respond surprisingly differently to these agents.

Figures 1 to 3 show that temozolomide, BCNU and fotemustine inhibit the growth of Raji cells in a dose-dependent manner but sensitivity is greatly increased by exposure to B.4316 at 0.1, 1.0 and 10 μ M respectively. In contrast B.4316 had no measurable effect on growth inhibition of Raji cells by melphalan or cisplatin (Fig. 4). This indicates that the inactivators were specifically sensitizing cells to the Q^6 -alkylating agents and not other classes of alkylating compound.

Figures 5 and 6 respectively show the B.4316 and B.4349 sensitization factors for the above therapeutic agents in Raji cells.

Figure 7 shows that of the inactivators examined human melanoma xenograft ATase depletion was complete only after administration of B.4314 and B.4351 under the experimental conditions used. The former was more effective in ATase depletion in liver and kidney of host animals whilst the latter was more effective in the brain, suggesting its relative ease in passing the blood-brain barrier. Noteworthy is the fact that whilst B.4311 was one of the most effective agents in sensitizing Raji cells to the

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toxic effects of temozolomide, it was surprisingly one of the least effective agents in depleting mouse tissue or tumour xenograft ATase activity.

5

Figure 8 shows that B.4363 depletes ATase more effectively in human melanoma xenografts than in murine host tissues under the conditions used: relatively little effect was seen in brain tissue, suggesting its poor ability to cross the blood brain barrier.

10

The test results for the compounds of Table 6 (and some in Table 1) are shown in Table 7 and Figures 9 to 27.

B.4280, which is O^6 -(4-bromophenyl)guanine and has its bromo
15 substituent in a 1, 3-relationship with the methylene group attached to the guanine residue, was more efficient in inactivating ATase *in vitro* than its 5-bromo analogue B.4269, in which the bromo substituent is in a 1, 4-relationship with the methylene group. Both B.4280 and B.4269 were more efficient than the unsubstituted
20 thenyl derivative B.4205 despite having considerably larger O^6 substituents.

Another preferred compound is B.4317 which is
 O^6 -(4-cyanophenyl)guanine. B.4317 is a more efficient inactivator
25 *in vitro* than its 5-cyano analogue B.4283 or the unsubstituted thenyl derivative B.4205.

Typical ATase inactivation profiles for BeG and B.4205 and B.4280 are shown in Fig. 9.

30

The inactivation of ATase resulted in the sensitization of Raji cells to the growth inhibitory effects of temozolomide (Fig. 10). B.4280 was considerably more effective than either B.4205 or BeG in this respect.

35

ATase in human melanoma xenografts was inactivated by BeG, B.4205 and B.4280 (Fig. 11) with some indication that the rates of

- 38 -

recovery of ATase activity were different between the agents. B.4280 was the most effective *in vivo* inactivator at the doses examined.

5

B.4280 was able to inactivate ATase in most tissues as shown in Table 8. Thus, activity in brain, testis and bone marrow was near to control levels by 24 hours whereas lung and spleen activity had not completely recovered by 48 hours. Tumour activity was very
10 low at 24 hours but had recovered completely by 48 hours. Differential recovery rates might be an important factor in the toxicity of ATase inactivators when used in combination with CNU or temozolomide.

15 Combinations of B.4205 or B.4280 and temozolomide given over three days were more effective in ATase inactivation in tumour xenografts than either agent alone (Fig. 12). Decreasing the dose of B.4205 had no major effect on the ability of the agent to inactivate ATase, 10mg/kg being as effective as 60mg/kg. B.4280 was more
20 effective than B.4205 at equivalent doses. As before (Fig. 11) there was some indication that ATase recovery was less efficient in the tumour xenograft (Fig. 12) than in the liver (Fig. 13).

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B.4205 (Fig. 14A) and B.4280 (Fig. 15A) were effective in sensitizing human melanoma xenografts to the growth inhibitory effects of temozolomide. A comparison of the two sets of data indicates that B.4280 was about twice as effective as B.4205 in this respect. At equi-effective doses for tumour growth inhibition, B.4280 seems to be less toxic than B.4205 (Figs. 14B and 15B).

In experiments using DBA₂ mice in combination with BCNU, B.4280 was considerably less acutely toxic than B.4205 or BeG as shown in Table 9. Oral administration of B.4280 was shown to be almost as effective as i.p. administration in sensitizing human melanoma xenografts to the growth inhibitory effects of temozolomide (Fig. 16A). Furthermore the oral combination appeared to be marginally less toxic than the i.p. route (Fig. 16B).

At a dose of 20mg/kg of inactivator in combination with temozolomide in DBA₂ mice, B.4205 and B.4280 were shown to be less acutely toxic than BeG, with B.4280 being less acutely toxic than B.4205 (Fig. 17).

Figures 18 and 19 show that B.4280 (PaTrin-2) (i.p. at 20 mg/kg and p.o at 30mg/kg respectively) depletes ATase in human melanoma xenografts more completely and for a more extensive period than it does in host tissues.

Figure 20 show that despite the considerably higher initial level of ATase activity in the human breast tumour, B.4280 depletes ATase therein more completely and for a longer period of time than in murine host tissues. In this study using 30mg/kg B.4280 i.p. extensive depletion was seen in brain tissue, indicating the ability to cross the blood-brain barrier.

Figure 21 likewise shows that despite the considerably higher initial level of ATase activity in the human prostate tumour, B.4280 depletes ATase therein more completely and for a longer period of time than in murine host tissues. In this study using 20mg/kg

- 40 -

B.4280 i.p. relatively little depletion was seen in brain tissue, indicating by reference to Figure 20 that the ability of B.4280 to cross the blood-brain barrier may be dose-dependent.

5

Figure 22 shows that B.4280 (20mg/kg i.p.) considerably increased the sensitivity of the human breast tumour xenograft to the growth inhibitory effects of temozolomide using a 5 day dosing schedule. This sensitization occurred despite the very high level of ATase in this tumour.

10

Figure 23 shows that a single dose of B.4280 (30mg/kg p.o.) considerably increased the sensitivity of the human melanoma tumour xenograft to the growth inhibitory effects of a single dose of the chloroethylating agent, fotemustine, without any substantial effect on toxicity.

15

Synthesis of Q^6 -(methylene[3H])-(4-bromophenyl)guanine

Bromophenylaldehyde (0.79mg, 66.8 umoles) was reacted with $NaB[^3H]_4$ (0.0167 mmoles, 60Ci/mmol) in isopropanol (350 μ l) for 1h at room temperature. The resulting [3H]-4-bromophenylalcohol was extracted into pentane, dried, weighed and reacted with NaH (5.44mg), and the quaternary ammonium salt of guanine (15.55mg) in DMSO (250 μ l) for 1 hour at room temperature. The product was recovered by precipitation from acetic acid-ether (15 μ l glacial acetic in 1.5ml ether), washed with ether, dried and triturated with H_2O . After washing with water, the final product was dried to constant weight. Figure 28 shows the scheme for synthesis of the radio-labelled B.4280.

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High performance liquid chromatography analysis

An aliquot of the product was dissolved in buffer A (10mM KH_2PO_4 containing 7.5% acetonitrile) and subjected to high performance liquid chromatography on an ODS-5 column. Elution at 1ml/min was with a linear gradient over 20 minutes from 100% A to 20% A:80% B (10mM KH_2PO_4 containing 80% acetonitrile). The effluent was monitored for UV absorption at 254nm and fractions (1

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min) were collected and assayed for radioactivity after addition of 10ml of Ecoscint A. It was shown that 96% of the radio activity co-chromatographed with authentic B.4280 (Figure 29).

5

Incubation of an aliquot of the product with known amounts of pure recombinant human ATase resulted in the transfer of radioactivity to the protein (Figure 30), strongly suggesting that the mechanism of ATase inactivation involves the transfer of the
10 thenyl group to the active site cysteine residue in the ATase molecule. Measurement of the amount of radioactivity transferred to protein indicated that the Q^6 -([^3H]-4-bromothienyl)guanine had a radiochemical purity of >96% and a specific activity of 16Ci/mmol.

15 Q^6 -([^3H]-4-bromothienyl)guanine can be used as an alternative to the standard method, which presently uses [^3H]-labelled substrate DNA, to determine the amounts of ATase, for example, in cell or tissue extracts. It may also be used to locate active ATase
20 molecules in tumour and other tissue sections by incubation with such sections on microscope slides followed by washing, autoradiography and histological staining. It may also be used to monitor the formation of the [^3H]-labelled products of breakdown or metabolism of the agent after administration to mammals. It may
25 also be used to determine the distribution of the B.4280 or its breakdown products in animal tissues and tumours by means of whole body autoradiography.

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Typical synthetic procedures

Type 1A.

O⁶-(4-Bromophenyl)hypoxanthine, B. 4292

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4-Bromophenyl alcohol (1.16 g, 6 mmol) was added to sodium hydride (60% in oil ; 0.16 g, 2 mmol) and DMSO (1 ml). The solution was stirred for 30 min. The trimethylammonium salt (0.427 g , 2 mmol) was then added and stirring continued for 2.5 h at 20°C. The solution was cooled in an ice bath and poured into
10 ether (60 ml) containing acetic acid (0.32 ml). A white precipitate was collected, triturated with water (4 ml) and collected again to give B. 4292 (436 mg , 69%) recrystallised from methanol.

Type 1B.

15

O⁶-Thenyl-2-methylhypoxanthine, B. 4350

DABCO salt from 6-chloro-2-methylpurine:

6-chloro-2-methylpurine (0.5 g, 3 mmol) was dissolved in a
20 mixture of DMF (5 ml) and diglyme (25 ml). DABCO (0.66 g, 6 mmol) was then added. The mixture was stirred for 1 h and the precipitate collected to give the quaternary salt (700 mg , 82%).

NMR (300 MHz, DMSO-d₆) : shift in ppm

2.65 (s), 3.27 (t, J=7.5 Hz), 3.78 (s), 4.14 (t, J=7.5 Hz), 8.21 (s).

25

Thenyl alcohol (684 mg , 6 mmol) was added to sodium hydride (60% in oil ; 80 mg , 2 mmol) and DMSO (0.5 ml). The solution was stirred for 30 min. The DABCO salt was then added and stirring continued for 5 h. The solution was then poured into ether (30 ml) containing acetic acid (0.15 ml). A precipitate was
30 collected, triturated with water (4 ml) and collected again to give O⁶-Thenyl-2-methylhypoxanthine (96 mg, 35%) recrystallised from acetonitrile.

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Type IC.*O*⁶-(4-Bromophenyl)-2-fluorohypoxanthine, B. 4353

5 To 3.6 ml of 40% fluoroboric acid precooled to -25°C in a bath was added *O*⁶-(4-bromophenyl) guanine (326 mg, 1 mmol) with vigorous stirring. A solution of sodium nitrite (0.116 g, 1.7 mmol) in water (0.15 ml) was added dropwise over a period of 10 min. After 20 min, the solution was poured into ice. The mixture was then allowed to stand at 0°C for 15 h, then collected and
10 dried to afford almost pure (t.l.c.) B. 4353 (180 mg, 55%). Flash chromatography (Hexane - Ethyl Acetate decreasing polarity little by little) afforded B. 4353 .

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Typical synthetic procedures (continued)**Type 3D****O⁴-Thenyl-5-deazapterin, B. 4376**

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a) N²-Pivaloyl-5-deazapterin

10 A mixture of 5-deazapterin^{33,34} (2.0g, 13.36mmol), 4-dimethylaminopyridine (0.22g, 1.8mmol) and pivalic anhydride (12ml) was heated to 165°C. Excess pivalic anhydride was distilled off and the residue dissolved in dichloromethane and applied to a pad of silica gel, and eluted with 2% methanol in dichloromethane. Evaporation and recrystallisation of the product from ethanol gave shiny cream coloured crystals (2.25g, 74%) of the pivaloyl derivative, m.p. 258-259°C; λ_{\max} (MeOH) 277 nm; NMR (300MHz, DMSO-d₆) δ 1.28(s), 7.44(q), 8.43(dd), 8.88(dd), 11.4(s), 12.31(s).

b) N²-pivaloyl-O⁴-thenyl-5-deazapterin :

15

20 A suspension of N²-pivaloyl-5-deazapterin (0.492g, 2mmol) in tetrahydrofuran (8ml) was stirred for 10 min, and tri-n-butylphosphine (0.606g, 3mmol), thenyl alcohol (0.432g, 3mmol) and diisopropylazodicarboxylate (0.606g, 3mmol) were added successively. The reaction was allowed to proceed for 2h at room temperature and evaporation then gave an oil. Hexane was added to induce crystallisation. Filtration and recrystallisation from hexane gave bright yellow crystals of the thenyl derivative (0.32g, 47%) m.p. 107-108°C; λ_{\max} (MeOH) 272, 311 nm; NMR (300MHz, DMSO-d₆) δ 1.28(s), 5.86(s), 6.98(q), 7.28(dd), 7.43(dd), 7.52(q), 8.46(dd), 8.89(dd).

c) B. 4376

25

N²-pivaloyl-O⁴-thenyl-5-deazapterin (0.28g, 0.82mmol) was heated for 24h under reflux with aqueous NaOH (3M, 2ml) and ethanol (1ml). The solvent was removed by evaporation and the residual solid dissolved in water. Acidification with acetic acid gave a white precipitate. Filtration and recrystallisation of the solid from ethanol gave white crystals of O⁴-thenyl-5-deazapterin (B. 4376), (0.107g, 51%).

Type 4D

30

O⁶-(4-Bromophenyl)-5-nitrocytosine, B.4380

35 Sodium hydride (60% in oil; 80mg, 2mmol) was added to a stirred solution of 4-bromophenyl alcohol (290mg, 1.5mmol) in dry DMSO (1ml). After 30 min, 4-amino-2-chloro-5-nitropyrimidine³⁵ (174mg, 1mmol) was added and the mixture heated at 50°C for 2h. The DMSO was removed in vacuo and the pH adjusted to 7 with aqueous acetic acid. After extraction into ethyl acetate, the product B. 4380 was crystallised from methanol (51mg, 15%).

Type 5*S⁶-(4-bromophenyl)-6-thioguanine*, B.4352

- 5 Sodium hydride (60% in oil; 44mg, 1.1mmol) was added to a stirred solution of 4-bromophenyl mercaptan (418mg, 2mmol) in dry DMSO (0.5ml). After 30 min, 2-amino-*N,N,N*-trimethyl-1*H*-purin-6-aminium chloride (228mg, 1mmol) was added and stirring continued for 1h. Acetic acid (0.12ml) and ether (30ml) were added and after decantation and trituration with fresh ether, B.4352 (38mg, 11%) was filtered off.

9-Substituted O⁶-(4-bromophenyl)guanines:

- 10 *O⁶-(4-Bromophenyl)-9-(ethoxymethyl)guanine*, B.4369

- 15 *O⁶-(4-Bromophenyl)guanine* (652mg, 2mmol) was dissolved in sodium ethoxide (1M; 2ml, 2mmol). After 10 min, the ethanol was removed and the residue was dissolved in dry DMF. Chloromethyl ethyl ether (189mg, 2mmol) was added dropwise to the stirred solution under an atmosphere of argon. After 45 min, the solvent was removed. The oily product was crystallised from ethanol giving B.4369 (158mg) as needles. A further 118mg was obtained by flash chromatography of the mother liquor on silica gel with 5% ethanol in CH₂Cl₂. Total yield, 39%.

O⁶-(4-Bromophenyl)-9-(2-hydroxyethoxymethyl)guanine, B. 4335

- 20 A stirred mixture of *O⁶-(4-bromophenyl)guanine* (294mg, 1mmol), (NH₄)₂SO₄ (47mg) and hexamethyldisilazane (5ml) was heated at reflux for 3h. Volatile material was then evaporated under vacuum. The residue was stirred with benzene (15ml) and Hg(CN)₂ (344mg, 1.3mmol) under reflux for 30 min. A solution of (2-acetoxyethoxy)methyl bromide {Ref 4 p33} (197mg, 1mmol) in benzene (10ml) was added, reflux maintained for 2h, and the cloudy diluted with chloroform (150ml). The organic phase was washed with saturated aqueous NaHCO₃ (30ml), followed by KI (1M ; 30ml), dried over MgSO₄ and evaporated to give an oil (313mg). This oil was chromatographed on a silica gel column with CHCl₃-MeOH (12 : 1) as eluant, yielding almost pure (t.l.c.) *O*-acetate (141mg) of B. 4335.

- 25 Methanol (60ml) was saturated with dry ammonia and poured onto this *O*-acetate in a flask which was tightly stoppered. After dissolution, stirring was stopped and the flask left closed overnight. Evaporation of methanol gave B. 4335 (135mg , 46%),
30 recrystallised from isopropanol.

O⁶-4-bromophenyl-9-(β-D-ribofuranosyl)guanine, B.4363

- 35 A mixture of 2',3',5'-tri-(*O*-acetyl)guanosine³⁶ (409mg, 1mmol), tri-*n*-butylphosphine (303mg, 1.5mmol) and 4-bromophenyl alcohol (290mg, 1.5mmol) in dry tetrahydrofuran (16ml) was stirred at room temperature for 45 min. Then diisopropyl azodicarboxylate (303mg, 1.5mmol) was added dropwise and the mixture stirred for 2h. The solution was evaporated leaving an oil which was dissolved in THF/MeOH/25% aqueous ammonia (1:1:1 ; 5 ml) and kept for 48 h at 4°C.

Adsorption on silica gel and column chromatography with $\text{CHCl}_3/\text{MeOH}$ (15:1 to 10:1) gave the riboside B.4363 (205mg, 44%).

*O*⁶-4-Bromothienyl-9-(β -D-2'-deoxyribofuranosyl)guanine, B.4379.

- 5 A mixture of 3',5'-di-(O-acetyl)-2'-deoxyguanosine³⁷ (554mg, 1.5mmol), tri-n-butylphosphine (666.6mg, 3.3mmol) and 4-bromothienyl alcohol (638mg, 3.3mmol) in dry tetrahydrofuran (40ml) was stirred at 80°C for 15 min. Then diisopropyl azodicarboxylate (666.6mg, 3.3mmol) was added dropwise and 15 min later, the reaction mixture was cooled and evaporated leaving an oil. This was dissolved in THF/MeOH/25% aqueous ammonia (1:1:1; 5 ml) and kept for 48 h at 4°C.
- 10 Adsorption on silica gel and column chromatography with $\text{CHCl}_3/\text{MeOH}$ (20:1) gave the 2'-deoxyriboside B.4379 (338mg, 51%).

9-(β -D-Arabinofuranosyl)-O⁶-(4-bromothienyl)guanine, B.4368.

- 15 An alkoxide solution was made from sodium hydride (60% in oil; 60mg, 1.5mmol) and 4-bromothienyl alcohol (344mg, 1.8mmol) in dry DMSO (0.5ml) over 1 h. It was reacted with 2-amino-9-(β -D-arabinofuranosyl)-6-chloropurine³⁸ (151mg, 0.5mmol) and stirred for 5 min at room temperature, then 15 min at 60-65°C. Cooling and trituration with ether (50ml) and filtration yielded a solid which was dissolved in water (5ml), neutralised with acetic acid and treated with silica gel. Column chromatography with ethyl acetate/MeOH (19:1) gave the arabinoside B.4368 (87mg, 38%), pure on t.l.c..

- 20 O⁶-substituted guanines

These were made by the standard procedure from the quaternary salt 2-amino-*N,N,N*-trimethyl-1*H*-purin-6-aminium chloride and the appropriate alkoxide derived from the alcohol and sodium hydride in DMSO (cf. pp. 16d, 17, 18, 47 of 7/12/95).

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TABLE 1A

| Compound, Test No. | O ⁶ -Substituent RCB ₂ | Yield % | Solvent for Recrystn. | M.p. (decomp.) (°C) | Formula | Molecular Weight | Analysis |
|--|---|------------|--------------------------|------------------------|---|---------------------|---|
| | | | | | | | C H N |
| Type 1A. Hypoxanthines | | | | | | | |
| B. 4293 | furfuryl | 60 | MeOH | 154 | C ₁₀ H ₈ N ₄ O ₂ | 216 | |
| B. 4291 | thenyl | 66 | MeOH | 168 | C ₁₀ H ₈ N ₄ OS | 232 | Found 51.85 3.40 24.12 Req. 51.71 3.47 24.12 |
| B. 4292 | 4-bromothienyl | 69 | MeOH | 170 | C ₁₀ H ₇ BrN ₄ OS | 311 | Found 38.33 2.18 17.66 Req. 38.6 2.26 18.00 |
| 1B. 2-Methylhypoxanthines | | | | | | | |
| B. 4347 | benzyl | 43 | MeCN | 191-193° | C ₁₃ H ₁₂ N ₄ O | 240 | Found 65.05 4.91 23.30 Req. 64.99 5.03 23.32 |
| B. 4350 | thenyl | 35 | MeCN | 176-178° | C ₁₁ H ₁₀ N ₄ OS | 325 | Found 53.63 3.90 22.67 Req. 53.64 4.09 22.75 |
| 1C. 2-Fluorohypoxanthines | | | | | | | |
| B. 4353 | 4-bromothienyl | 55 | Column | 142 | C ₁₀ H ₆ BrFN ₄ OS | 329 | |
| 1D. 9-(2-Hydroxyethoxy- methyl)guanines | | | | | | | |
| B. 4334 | benzyl | 46 | i-PrOH | 150-152° | C ₁₅ H ₁₇ N ₅ O ₃ | 315 | Found 57.19 5.59 21.93 Req. 57.13 5.43 22.21 |
| B. 4335 | 4-bromothienyl | 42 | i-PrOH | 156-158° | C ₁₃ H ₁₄ BrN ₅ O ₃ S | 400 | Found 39.16 3.68 17.20 Req. 39.01 3.53 17.50 |
| 1E. 8-Hydroxyguanines | | | | | | | |
| B. 4349 | 4-bromothienyl | 56 | Aq. EtOH | > 230 | C ₁₀ H ₆ BrN ₅ O ₃ S · ½ H ₂ O | 351 | Found 34.53 2.48 19.50 Req. 34.20 2.58 19.94 |
| Type 2A. 8-Azaguanines | | | | | | | |
| B. 4270 | 4-fluorobenzyl | 40 | Aq. MeOH | > 280 | C ₁₁ H ₉ FN ₄ O | 260 | Found 51.50 3.85 29.44 Req. 50.77 3.49 32.30 |
| B. 4314 | 4-chlorothienyl | 26 | Aq. MeOH | > 200 | C ₈ H ₇ ClN ₄ OS | 282.7 | Found 38.86 2.61 28.61 Req. 38.24 2.50 29.73 |
| B. 4289 | 4-bromothienyl | 12 | MeCN | > 190 | C ₉ H ₇ BrN ₄ OS | 327 | Found 35.91 2.78 24.60 |

TABLE 1A (continued)

| Compound, Test No. | O ⁶ -Substituent RCH ₃ | Yield % | Solvent for Recrystn. | M.p. (decomp.) (°C) | Formula | Molecular Weight | | C | H | N |
|--|---|------------|--------------------------|------------------------|--|---------------------|-------|-------|------|-------|
| | | | | | | | Req. | 33.04 | 2.16 | 25.69 |
| 2B. 8-Aza-7-deazaguanines B. 4310 B. 4340 B. 4339 B. 4343 B. 4348 B. 4338 B. 4337 | benzyl | | MeOH | 160 | C ₁₂ H ₁₁ N ₅ O ₂ H ₂ O | 259 | Found | 55.53 | 4.9 | 26.41 |
| | 4-fluorobenzyl | 65 | EtOH | 188 | C ₁₂ H ₁₀ FN ₅ O ₂ ·½H ₂ O | 263.7 | Req. | 55.59 | 5.01 | 27.02 |
| | 4-chlorobenzyl | 92 | EtOH | 242-244° | C ₁₂ H ₁₀ ClN ₅ O ₂ ·½H ₂ O·½EtOH | 296 | Found | 53.82 | 3.76 | 26.97 |
| | piperonyl | 50 | EtOH | 186 | | | Req. | 54.6 | 4.0 | 26.55 |
| | furfuryl | | EtOH | 150° | C ₁₃ H ₁₁ N ₅ O ₃ | 285 | Found | 54.52 | 3.82 | 24.50 |
| | thenyl | 68 | EtOH | 180 | C ₁₀ H ₉ N ₅ O ₂ ·½H ₂ O | 235.7 | Req. | 54.7 | 3.88 | 24.55 |
| | 4-bromothienyl | 79 | EtOH | 180 | C ₁₀ H ₉ N ₅ OS | 247 | Found | 50.96 | 3.87 | 29.54 |
| | | | | | C ₁₀ H ₉ BrN ₅ OS | 326 | Req. | 50.96 | 4.06 | 29.71 |
| Type 3A. 8-Oxaguanines B. 4272 B. 4285 B. 4299 B. 4287 | 4-fluorobenzyl | 41 | Acetone | 223-224 | C ₁₁ H ₉ FN ₅ O ₂ | 261 | Found | 47.58 | 3.54 | 27.41 |
| | 4-chlorobenzyl | 63 | Acetone | 219-220 | C ₁₁ H ₈ ClN ₅ O ₂ | 277.7 | Req. | 47.7 | 3.8 | 27.8 |
| | 4-chlorothienyl | 55 | Acetone | 164-165 | C ₉ H ₆ ClN ₅ O ₂ S | 283.7 | Found | 37.08 | 2.51 | 21.34 |
| | 4-bromothienyl | 61 | Acetone | 170-172 | C ₉ H ₆ BrN ₅ O ₂ S | 328 | Req. | 36.8 | 2.5 | 21.5 |
| | | | | | | | Found | 50.39 | 3.08 | 26.65 |
| 3B. 8-Thioguanines B. 4296 B. 4286 | benzyl | 39 | EtOH | | C ₁₁ H ₉ N ₅ OS | 259 | Req. | 50.58 | 3.09 | 26.81 |
| | 4-fluorobenzyl | 11 | PLC | 182-184 | C ₁₁ H ₈ FN ₅ OS | 277 | Found | 47.59 | 2.88 | 25.25 |
| | | | | | | | Req. | 47.58 | 2.90 | 25.22 |
| | | | | | | | Found | 37.68 | 2.15 | 24.43 |
| | | | | | | | Req. | 38.10 | 2.13 | 24.69 |
| | | | | | | | Found | 33.30 | 1.85 | 21.37 |
| | | | | | | | Req. | 32.94 | 1.84 | 21.34 |

TABLE 1A (continued)

| Compound, Test No. | O ⁶ -Substituent RCH ₂ | Yield % | Solvent for Recrystn. | M.p. (decomp.) (°C) | Formula | Molecular Weight | | C | H | N |
|--|---|------------|---------------------------------------|------------------------|---|---------------------|-------|-------|------|-------|
| B. 4315 | 4-chlorophenyl | 13 | MeOH | | C ₉ H ₆ ClN ₃ OS ₂ | 299.8 | Found | 36.27 | 2.04 | 23.07 |
| B. 4351 | 4-bromophenyl | 41 | MeOH | 156-160 | C ₉ H ₆ BrN ₃ OS ₂ | 344 | Req. | 36.06 | 2.02 | 23.36 |
| | | | | | | | Found | 31.49 | 1.60 | 20.11 |
| | | | | | | | Req. | 31.41 | 1.76 | 20.35 |
| 3C. <i>Pterins</i> (O ⁴ -substituent) | | | | | | | | | | |
| B. 4290 | 4-fluorobenzyl | 55 | MeOH | >210 | C ₁₃ H ₁₀ FN ₃ O | 271 | Found | 57.87 | 3.88 | 25.65 |
| B. 4316 | 4-chlorophenyl | 41 | MeOH | >170 | C ₁₁ H ₆ ClN ₃ OS | 293.7 | Req. | 57.56 | 3.72 | 25.82 |
| B. 4288' | 4-bromophenyl | 63 | MeOH | 178-179 | C ₁₁ H ₆ BrN ₃ OS | 338 | Found | 44.98 | 2.75 | 23.84 |
| | | | | | | | Req. | 39.34 | 3.13 | 20.25 |
| | | | | | | | Req. | 39.07 | 2.38 | 20.71 |
| Type 4A. 2,4-diamino-6-hydroxypyrimidines | | | | | | | | | | |
| B. 4305 | 4-fluorobenzyl | 98 | C ₆ H ₆ /Petrol | 133-134 | C ₁₁ H ₁₁ FN ₄ O | 234 | Found | 56.20 | 4.79 | 23.66 |
| B. 4304 | 4-chlorobenzyl | 31 | C ₆ H ₆ | 122-123 | C ₁₁ H ₁₁ ClN ₄ O | 250.7 | Req. | 56.40 | 4.73 | 23.92 |
| B. 4303 | piperonyl | 79 | MeCN | 168-171 | C ₁₂ H ₁₃ N ₄ O ₃ | 260 | Found | 52.43 | 4.56 | 22.47 |
| B. 4307 | thienyl | 97 | MePh | 100 | C ₉ H ₁₀ N ₄ OS | 222 | Req. | 52.70 | 4.42 | 22.35 |
| B. 4302 | 4-chlorophenyl | 45 | MePh | 129-130 | C ₉ H ₆ ClN ₄ OS | 256.7 | Found | 55.31 | 4.64 | 21.38 |
| | | | | | | | Req. | 55.38 | 4.65 | 21.52 |
| | | | | | | | Found | 48.83 | 4.58 | 25.25 |
| | | | | | | | Req. | 48.63 | 4.54 | 25.21 |
| | | | | | | | Found | 42.40 | 3.68 | 22.00 |
| | | | | | | | Req. | 42.11 | 3.53 | 21.83 |
| 4B. 2,4-Diamino-6-hydroxy-5-nitrosopyrimidines | | | | | | | | | | |
| B. 4301 | 4-fluorobenzyl | 76 | MeOH | >250 | C ₁₁ H ₁₀ FN ₃ O ₂ | 263 | Found | 49.60 | 3.90 | 26.29 |
| B. 4311 | 4-chlorophenyl | 84 | Acetone | >190 | C ₉ H ₆ ClN ₃ O ₂ S | 285.7 | Req. | 50.19 | 3.83 | 26.61 |
| | | | | | | | Found | 37.54 | 2.79 | 24.22 |

TABLE 1A (continued)

| Compound, Test No. | O ⁶ -Substituent RCH ₃ | Yield % | Solvent for Recrystn. | M.p. (decomp.) (°C) | Formula | Molecular Weight | Analysis | | | |
|---|---|------------|--------------------------|------------------------|--|---------------------|--------------------------------|------------------------------|----------------------------------|--|
| | | | | | | | C | H | N | |
| B. 4312 | 4-bromothienyl | 62 | Acetone | 200-201 | C ₉ H ₈ BrN ₃ O ₃ S | 330 | Req. Found | 2.82 2.38 | 24.51 20.96 | |
| 4C. 2,4-Diamino-6-hydroxy-5-nitropyrimidines B. 4308 | | | | | | | Req. | 2.44 | 21.21 | |
| B. 4306 | piperonyl thienyl | 67 34 | DMF MeOH | >175 159-160 | C ₁₂ H ₁₁ N ₃ O ₃ C ₈ H ₆ N ₄ O ₃ S | 305 267 | Found Req. Found Req. | 4.07 3.63 3.71 3.39 | 22.83 22.94 25.99 26.21 | |

Continuation of Table 1a

| Compound, Test No. | O ⁶ -Substituent RCH ₃ | Yield % | Solvent for Recrystn. | M.p. (decomp.) (°C) | Formula | Molecular Weight | Analysis | | | |
|--|---|------------|--|------------------------|--|---------------------|--------------------------------|----------------------------------|------------------------------|----------------------------------|
| | | | | | | | C | H | N | |
| Type 3D. 5-Deazapterins (O ⁶ -substituent) B. 4376 | thienyl | 51 | EtOH | 215-216 | C ₁₂ N ₁₀ N ₄ OS | 258 | | | | |
| 4D. 5-Nitrocyasines (O ⁶ -substituent) B. 4380 | 4-bromothienyl | 15 | MeOH | 143-144 | C ₈ H ₇ BrN ₄ O ₃ S | 331 | | | | |
| 5. 6-Thioguanines (S ⁶ -substituent) B. 4228 B. 4352 | piperonyl 4-bromothienyl | 69 11 | CH ₃ OH CH ₃ OH | 204-212 180-184 | C ₁₃ H ₁₁ N ₃ O ₃ S C ₁₀ H ₄ BrN ₃ O ₃ CH ₃ OH | 301 342 | Found Req. Found Req. | 50.25 51.82 35.07 35.16 | 3.60 3.68 2.42 2.66 | 23.66 23.24 19.49 19.84 |

TABLE 1A (continued)

| Compound, Test No. | 9-Substituent | Yield % | Solvent for Recrystn. | M.p. (°C) | Formula | Molecular Weight | Analysis ^a | | |
|---------------------|-------------------------------|---------|-----------------------|--------------|---|---------------------|-----------------------|--------------|-----------------|
| | | | | | | | C | H | N |
| B.4369 | ethoxymethyl | 39 | EtOH | 134-5 | C ₁₃ H ₁₄ BrN ₃ O ₂ S | 384 | 40.58 (40.64) | 3.71 3.67 | 17.97 18.23) |
| B.4370 | <i>n</i> -octyloxymethyl | 39 | EtOH | 90 | C ₁₉ H ₂₆ BrN ₃ O ₂ S | 468 | 48.97 (48.72) | 5.67 5.60 | 14.82 14.95) |
| B.4334 ^b | 2-hydroxy- ethoxymethyl | 46 | <i>i</i> -PrOH | 150-2 | C ₁₅ H ₁₇ N ₃ O ₃ | 315 | 57.19 (57.13) | 5.59 5.43 | 21.93 22.21) |
| B.4335 | 2-hydroxy- ethoxymethyl | 42 | <i>i</i> -PrOH | 156-8 | C ₁₃ H ₁₄ BrN ₃ O ₃ S | 400 | 39.16 (39.01) | 3.68 3.53 | 17.20 17.50) |
| B.4363 | β-D-ribo- furanosyl | 44 | | | C ₁₃ H ₁₆ BrN ₃ O ₃ S | 458 | | | |
| B.4368 | β-D-arabino- furanosyl | 38 | | | C ₁₃ H ₁₆ BrN ₃ O ₃ S | 458 | | | |
| B.4379 | β-D-2-deoxyribo- furanosyl | 51 | | | C ₁₃ H ₁₆ BrN ₃ O ₄ S | 442 | | | |

^a Found, with required values
in parenthesis.^b *O*⁶-benzyl

TABLE 1B

| Compound Type, Test No. | <i>O</i> ⁶ -Substituent RCH ₂ | λ_{max} (nm) | δ_H (ppm from TMS, (CD ₃) ₂ SO- <i>d</i> ₆) (Hz) |
|--|---|----------------------|--|
| Type 1A. Hypoxanthines | | | |
| B. 4293 | furfuryl | 252 | 5.60(s), 6.53(dd, 3.1, 1.9), 6.69(d, 3.1), 7.76(dd, 1.9, 0.9) 8.39(s), 8.55(s) |
| B. 4291 | thenyl | 240 | 5.83(s), 7.08(dd, 5.1, 3.4), 7.35(d, 3.4), 7.6(d, 5.1), 8.39(s), 8.51(s) |
| B. 4292 | 4-bromothienyl | 251 | 5.80(s), 7.38(d, 1.3), 7.73(d, 1.3), 8.42(s), 8.58(s) |
| Type 1B. 2-Methylhypoxanthines | | | |
| B. 4347 | benzyl | 256 | 2.61(s), 5.60(s), 7.50(m), 8.32(s) |
| B. 4350 | thenyl | 240 | 2.63(s), 5.77(s), 7.05(dd, 5.1, 2.4, 7.33(d, 2.4), 7.58(dd, 5.1, 1.0) 8.26(s), 13.22(s) |
| Type 1C. 2-Fluorohypoxanthines | | | |
| B. 4353 | 4-bromothienyl | 233, 255 | 5.77(s), 7.4(d, 1.5), 7.77(d, 1.5), 8.45(s), 13.64(bs) |
| Type 1D. 9-(2-Hydroxyethoxymethyl)guanines | | | |
| B. 4334 | benzyl | 247, 283 | 3.48(m), 4.70(s), 5.45(s), 6.59(s), 7.45(m), 8.03(s), |
| B. 4335 | 4-bromothienyl | 245, 284 | 3.49(m), 4.71(s), 5.45(s), 5.66(s), 6.65(s), 7.30(d, 1.5) 7.72(d, 1.5) 8.04(s) |
| Type 1E. 8-Hydroxyguanines | | | |
| B. 4349 | 4-bromothienyl | 239, 293 | 5.54(s), 6.24(s), 7.33(d, 1.4) 7.70(d, 1.4), 10.49(s) 11.12(s) |
| Type 2A. 8-Azaguanines | | | |
| B. 4270 | 4-fluorobenzyl | 288 | 5.57(s), 7.04(s), 7.28(m), 7.65(m), 15.38(s) |
| B. 4314 | 4-chlorothienyl | 288 | 5.71(s), 7.13(s), 7.41(d, 1.5), 7.66(d, 1.5), 15.42(s) |
| B. 4289 | 4-bromothienyl | 287 | 5.73(s), 7.12(s), 7.43(d, 1.5), 7.76(d, 1.5), 15.39(s) |

TABLE 1B (continued)

| | | | |
|---|-----------------|----------------------|---|
| Type 2B, 8-Aza-7-deazaquinines | | | |
| B. 4310 | benzyl | 277 | 5.50(s), 6.68(s), 7.74(m), 7.82(s), 12.87(bs) |
| B. 4340 | 4-fluorobenzyl | 278 | 5.49(s), 6.70(s), 7.20(m), 7.61(m), 7.82(s), 12.88(bs) |
| B. 4339 | 4-chlorobenzyl | 276 | 5.50(s), 6.69(s), 7.49(d, 8.4), 7.56(d, 8.4), 7.83(s), 12.90(s) |
| B. 4343 | piperonyl | 282 | 5.39(s), 6.05(s), 6.69(s), 6.94(d, 7.9), 7.04(dd, 7.9, 1.5), 7.14(d, 1.5), 7.80(s), 12.86(bs) |
| B. 4348 | furfuryl | 277 | 5.46(s), 6.52(s), 6.70(s), 6.71(s), 7.73(s), 7.79(s), 12.85(bs) |
| B. 4338 | thienyl | 278 | 5.69(s), 6.73(s), 7.07(d, 3.5), 7.35(s), 7.60(d, 1.1), 7.79(s), 12.90(bs) |
| B. 4337 | 4-bromothienyl | 278 | 5.65(s), 6.76(s), 7.38(s), 7.72(d, 1.3), 7.81(s), 12.91(bs) |
| Type 3A, 8-Oxaquinines | | | |
| B. 4272 | 4-fluorobenzyl | 257, 341 | 5.62(s), 7.30(t, 9.1), 7.68(m), 7.91(s), 7.97(s) |
| B. 4285 | 4-chlorobenzyl | 256, 340 | 5.63(s), 7.53(d, 8.3), 7.65(d, 8.3), 7.90(s), 7.97(s) |
| B. 4299 | 4-chlorothienyl | 252, 343 | 5.78(s), 7.46(d, 1.6), 7.72(d, 1.6), 7.95(s), 8.01(s) |
| B. 4287 | 4-bromothienyl | 253, 343 | 5.79(s), 7.49(d, 1.6), 7.81(d, 1.6), 7.95(s), 8.01(s) |
| Type 3B, 8-Thiaquinines | | | |
| B. 4296 | benzyl | 227, 361 | 5.59(s), 7.29(t, 8.9), 7.51(bs), 7.67(m) |
| B. 4286 | 4-fluorobenzyl | 235, 362 | 5.75(s), 7.44(d, 1.6), 7.55(bs), 7.69(d, 1.6) |
| B. 4315 | 4-chlorothienyl | 228, 360 | 5.78(s), 7.45(d, 1.6), 7.46(bs), 7.75(d, 1.6) |
| B. 4351 | 4-bromothienyl | 228, 361 | |
| Type 3C, <i>Pterins</i> (<i>O'</i> -substituent) | | | |
| B. 4200 | 4-fluorobenzyl | 232, 264(sh), 362 | 5.56(s), 7.29(t, 8.85), 7.44(bs), 7.66(m), 8.45(d, 1.8), 8.82(d, 1.8) |
| B. 4316 | 4-chlorothienyl | 232, 364 | 5.71(s), 7.41(d, 1.6), 7.47(bs), 7.67(d, 1.6), 8.46(d, 2.0), 8.83(d, 2.0) |
| B. 4288 | 4-bromothienyl | 231, 364 | 5.73(s), 7.44(d, 1.6), 7.50(bs), 7.77(d, 1.6), 8.46(d, 2), 8.83(d, 2) |

TABLE 18 (continued)

| | | | |
|--|----------------|----------|--|
| Type 4A. 2,4-diamino-6-hydroxypyrimidines | | | |
| B. 4305 | 4-fluorobenzyl | 238, 267 | 5.10(s), 5.19(s), 5.96(s), 6.10(s), 7.19(t, 8.8), 7.44(dd, 8.8, 5.8). |
| B. 4304 | 4-chlorobenzyl | 238, 268 | 5.11(s), 5.22(s), 5.96(s), 6.10(s), 7.44(s). |
| B. 4303 | piperonyl | 236, 267 | 5.09(s), 5.11(s), 5.97(s), 6.03(s), 6.07(s), 6.91(d, 1.1), 7.00(s). |
| B. 4307 | thenyl | 235, 267 | 5.08(s), 5.40(s), 6.00(s), 6.10(s), 7.03(dd, 8.1, 3.5) 7.20(dd, 8.1, 1.1), 7.54(dd, 3.5, 1.1). |
| B. 4302 | 4-chlorothenyl | 236, 265 | 5.08(s), 5.35(s), 6.03(s), 6.13(s), 7.19(s), 7.55(d 1.6). |
| Type 4B. 2,4-diamino-6-hydroxy-5-nitrooxypyrimidines | | | |
| B. 4301 | 4-fluorobenzyl | 336 | 5.59(s), 7.26(m), 7.65(m), 7.80(bs), 7.85(bs), 8.00(bs), 10.05(bs). |
| B. 4311 | 4-chlorothenyl | 335 | 5.73(s), 7.40(d, 1.6), 7.66(d, 1.6), 7.94(s), 7.98(d, 2.7), 8.11(d, 4.2) 10.03(d, 4.2). |
| B. 4312 | 4-bromothenyl | 335 | 5.75(s), 7.42(d, 1.4), 7.75(d, 1.4), 7.93(s), 7.98(s), 8.12(d, 4.0), 10.04(d, 4.0). |
| Type 4C. 2,4-diamino-6-hydroxy-5-nitroprymidines | | | |
| B. 4308 | piperonyl | 288, 330 | 5.33(s), 6.05(s), 6.95(d, 8.0), 7.00(dd, 8.0, 1.4), 7.10(d, 1.4); 7.26(bs), 7.37.96(bs). |
| B. 4306 | thenyl | 234, 329 | 5.59(s), 7.03(dd, 5.1, 3.5), 7.28(d, 3.5), 7.32(bs), 7.56(d, 5.1), 7.94(bs). |

TABLE 1B (continued)

| Compound Type, Test No. | Substituent RCH ₂ | λ_{max} (MeOH) (nm) | δ_{H} [ppm from TMS, (CD ₃) ₂ SO-d ₆]/J(Hz) |
|---|---------------------------------|---------------------------------------|--|
| Type 3D 5-Deazapterins (<i>O'</i>-substituent) | | | |
| B 4376 | thenyl | 248, 309 | 5.54(s), 6.96(q), 7.716(dd), 7.38(dd), 7.41(q), 8.39(dd), 8.79(dd). |
| Type 4D 5-Nitrocytosines (<i>O'</i>-substituent) | | | |
| B 4380 | 4-bromothienyl | 255 sh, 334 | 5.19(s), 7.20(s), 7.56(d), 8.24(s), 8.70(s), 8.90(s). |
| Type 5 6-Thioguanines (<i>S'</i>-substituent) | | | |
| B 4228 | piperonyl | 245, 311 | 4.56(s), 6.06(s), 6.55(s), 7.03(d), 7.06(d), 7.14(s), 8.08(s), 12.67(bs). |
| B 4352 | 4-bromothienyl | 241, 314 | 4.77(s), 6.52(s), 7.18(d), 7.51(d), 7.93(s), 12.61(bs). |

TABLE 1B (continued)

| Compound Type, Test No. | 9-Substituent | Yield % | λ_{max} (MeOH) (nm) | δ_{H} ppm from TMS, $(\text{CD}_3)_2\text{SO}-d_6$ (Hz) |
|-------------------------|----------------------------------|---------|---------------------------------------|---|
| B.4369 | ethoxymethyl | | 245, 284 | 3.35(s), 5.41(s), 5.66(s), 6.66(s), 7.38(d), 7.73(d), 8.04(s). |
| B.4370 | <i>n</i> -octyloxymethyl | | 245, 284 | 0.09(t), 1.17(m), 3.36(t), 5.41(s), 5.66(s), 6.66(s), 7.38(d), 7.72(d), 8.03(s). |
| B.4334 a | 2-hydroxy-ethoxymethyl | | 245, 283 | 3.48(m), 4.70(s), 5.45(s), 6.59(s), 7.45(s), 8.03(s). |
| B.4335 | 2-hydroxy-ethoxymethyl | | 245, 284 | 3.49(m), 4.71(s), 5.45(s), 5.66(s), 6.65(s), 7.30(d, 1.5), 7.72(d, 1.5), 8.40(s). |
| B.4363 | β -D-ribo-furanosyl | | - | 3.54(m), 3.63(m), 3.91(dd), 4.12(dd), 4.48(ddd), 5.12(dd), 5.18(d), 5.45(d), 5.66(s), 5.80(dd), 6.61(s), 7.38(d), 7.71(d), 8.15(s). |
| B.4368 | β -D-arabino-furanosyl | | 245, 284 | 3.64(m), 3.76(dd), 4.07(m), 5.09(dd), 5.51(d), 5.53(m), 6.13(d), 6.60(d), 7.37(d), 7.71(d), 7.95(s). |
| B.4379 | β -D-2-deoxyribo-furanosyl | | | 2.39(ddd), 2.72(ddd), 3.65(ddd), 3.98(dd), 4.40(dd), 5.11(s), 5.41(d), 5.80(s), 6.38(dd), 6.67(s), 7.49(d), 7.83(d), 8.25(s). |

^a
06-benzyl

| TABLE 2 | | I ₅₀ (μM) hAT | T 1/2 (h) in PBS |
|------------------|---|-----------------------------|---------------------|
| INACTIVATOR TYPE | | | |
| 5 | 1A | | |
| | B.4291 O ⁶ -(thenyl)-hypoxanthine | 1.9 | >20 |
| | B.4293 O ⁶ -(furfuryl)-hypoxanthine | 28 | >16 |
| 10 | B.4292 O ⁶ -(4-bromothernyl)-hypoxanthine | 0.3 | >16 |
| | O ⁶ -(benzyl)-hypoxanthine b | 85 | |
| | 1B | | |
| 15 | B.4347 O ⁶ -(benzyl)-2-methylhypoxanthine | 75 | |
| | B.4350 O ⁶ -(thenyl)-2-methylhypoxanthine | 14 | |
| | 1C | | |
| 20 | B.4353 O ⁶ -(4-bromothernyl)-2-fluorohypoxanthine | 1.4 | |
| | O ⁶ -(benzyl)-2-fluorohypoxanthine a | 48 | |
| | 1D | | |
| 25 | B.4334 O ⁶ -(benzyl)-9-(2-hydroxyethoxymethyl) guanine | 8 | >20 |
| | B.4335 O ⁶ -(4-bromothernyl)-9-(2-hydroxy ethoxymethyl)guanine | See Table 3 | |
| 30 | 1E | | |
| | B.4349 O ⁶ -(4-bromothernyl)-8-hydroxyguanine | See Table 3 | |
| | O ⁶ -(benzyl)-8-hydroxyguanine a | 0.3 | |
| 35 | 2A | | |
| | B.4270 O ⁶ -(4-fluorobenzyl)-8-azaguanine | 0.08 | |

TABLE 2 (continued)
INACTIVATOR TYPE

| | | I₅₀(μM) hAT | T 1/2 (h) in PBS |
|----|--|--|-----------------------------|
| 5 | B.4314 <u>0</u> ⁶ -(4-chlorothenyl)-8-azaguanine | See Table 3 | |
| | B.4289 <u>0</u> ⁶ -(4-bromothenyl)-8-azaguanine | 0.045 | >10 |
| | <u>0</u> ⁶ -(benzyl)-8-azaguanine ^a | 0.07 | |
| 10 | 28 | | |
| | B.4310 <u>0</u> ⁶ -(benzyl)-7-deaza-8-azaguanine | 0.01 | >16 |
| | B.4340 <u>0</u> ⁶ -(4-fluorobenzyl)-8-aza-7-deazaguanine | 0.018 | >16 |
| 15 | B.4339 <u>0</u> ⁶ -(4-chlorobenzyl)-8-aza-7-deazaguanine | 0.02 | 1.5 |
| | B.4343 <u>0</u> ⁶ -(piperonyl)-8-aza-7-deazaguanine | See Table 3 | |
| 20 | B.4348 <u>0</u> ⁶ -(furfuryl)-8-aza-7-deazaguanine | 0.036 | 0.27 |
| | B.4338 <u>0</u> ⁶ -(thenyl)-8-aza-7-deazaguanine | 0.01 | |
| | B.4337 <u>0</u> ⁶ -(4-bromothenyl)-8-aza-7-deazaguanine | 0.007 | >20 |
| 25 | 3A | | |
| | B.4272 <u>0</u> ⁶ -(4-fluorobenzyl)-8-oxaguanine | See Table 3 | |
| | B.4285 <u>0</u> ⁶ -(4-chlorobenzyl)-8-oxaguanine | 0.225 | 4.6 |
| 30 | B.4299 <u>0</u> ⁶ -(4-chlorothenyl)-8-oxaguanine | 0.243 | 9.2 |
| | B.4287 <u>0</u> ⁶ -(4-bromothenyl)-8-oxaguanine | 0.24 | 2.6 |
| 35 | B.4232 <u>0</u> ⁶ -(benzyl)-8-oxaguanine | 0.25 | |

| TABLE 2 (continued) | | I ₅₀ (μ M) hAT | T 1/2 (h) in PBS |
|---------------------|--|-----------------------------------|---------------------|
| INACTIVATOR TYPE | | | |
| | 3B | | |
| 5 | B.4296 <u>0</u> ⁶ -(benzyl)-8-thiaguanine | 0.02 | >17 |
| | B.4286 <u>0</u> ⁶ -(4-fluorobenzyl)-8-thiaguanine | 0.03 | >17 |
| 10 | B.4315 <u>0</u> ⁶ -(4-chlorothenyl)-8-thiaguanine c | 0.006 | |
| | B.4351 <u>0</u> ⁶ -(4-bromothenyl)-8-thiaguanine | See Table 3 | |
| | 3C | | |
| 15 | B.4290 <u>0</u> ⁴ -(4-fluorobenzyl)-pterin | 0.088 | >10 |
| | B.4316 <u>0</u> ⁴ -(4-chlorothenyl)-pterin | See Table 3 | |
| | B.4288 <u>0</u> ⁴ -(4-bromothenyl)-pterin | 0.025 | >10 |
| 20 | 4A | | |
| | B.4305 2,4-diamino-6-(4-fluorobenzyloxy)pyrimidine | 4.0 | >16 |
| 25 | B.4304 2,4-diamino-6-(4-chlorobenzyloxy)pyrimidine | 5.0 | >16 |
| | B.4303 2,4-diamino-6-(3,4-piperonyloxy)pyrimidine | 0.8 | 12.5 |
| | B.4307 2,4-diamino-6-(thenyloxy)pyrimidine | 0.4 | 4.2 |
| 30 | B.4302 2,4-diamino-6-(4-chlorothenyloxy)pyrimidine | 0.17 | >16 |
| | 2,4-diamino-6-(benzyloxy)pyrimidine a | 15 | |
| | 4B | | |
| 35 | B.4301 2,4-diamino-6-(4-fluorobenzyloxy)-5-nitrosopyrimidine | 0.0175 | >16 |

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TABLE 2 (continued)
INACTIVATOR TYPE

| | | I₅₀(uM) hAT | T 1/2 (h) in PBS |
|----|---|-----------------------------------|-----------------------------|
| 5 | B.4311 2,4-diamino-(4-chlorothenyloxy)-5-nitrosopyrimidine | See Table 3 | |
| | B.4312 2,4-diamino-6-(4-bromothenyloxy)-5-nitrosopyrimidine | 0.045 | 4 |
| 10 | 2,4-diamino-6-(benzyloxy)-5-nitrosopyrimidine ^a | 0.06 | |
| | 4C | | |
| | B.4306 2,4-diamino-6-(thenyloxy)-5-nitropyrimidine | 2.3 | >16 |
| 15 | B.4308 2,4-diamino-6-piperonyloxy-5-nitropyrimidine | 0.5 | 9.2 |
| | 2,4-diamino-6-benzyloxy-5-nitropyrimidine ^a | 0.06 | |
| | 4D | | |
| 20 | B.4380 <u>0</u> ² (4-bromotheryl)-5-nitrocytosine | 50 | |
| | 5 | | |
| | B.4228 <u>S</u> ⁶ -(piperonyl)-6-thioguanine | 50 | |
| 25 | B.4352 <u>S</u> ⁶ -(4-bromotheryl)-6-thioguanine | 8 | |
| | Comparative | | |
| 30 | B.4376 <u>0</u> ⁶ -thenyl-5-deazapterin | 1,600 | |
| | Results for some 9-substituted <u>0</u> ⁶ (4-bromotheryl)guanines are included in Table 7. | | |
| 35 | a Data taken from Chae et al, <u>J. Med. Chem.</u> 1995, 38, 359-365 b Data taken from Moschel et al., <u>J. Med. Chem.</u> 1992, 35, 4486-4491. c B.4315 Raji I ₅₀ (uM) 0.002 | | |

Blank Space = not done.

TABLE 3

| Inactivator | Mol Wgt | I ₅₀ hAT (µM) | I ₅₀ Raji (µM) | I ₅₀ mAT (µM) | I ₅₀ rAT (µM) | I ₅₀ chAT (µM) | I ₅₀ ogt (µM) | I ₅₀ ada (µM) | T ^{1/2} (h) PBS | T ^{1/2} (h) by Assay | Raji cell sensitisation factor (D ₅₀ control / D ₅₀ 'B') | | | | | Solubility in Water (mg/ml) | Raji cell toxicity at 10 µM 'B' alone (% Growth) |
|--------------------|------------|--------------------------------|------------------------------|--------------------------------|--------------------------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------------|---|--------------------------------|------|-----|-------|-----------------------------------|--|
| | | | | | | | | | | | BCNU | TEMOZOLOMIDE | | | | | |
| | | | | | | | | | | | | Inactivator concentration (µM) | | | | | |
| | | | | | | | | | | | | 10 | 10 | 1.0 | 0.5 | | |
| B4272 | 261 | 0.05 | 0.023 | 0.125 | 0.075 | 0.04 | >1000 | >1000 | 5.7 | 2.6 | 1.88 | 1.41 | --- | --- | 0.002 | 111.21±23.3 | |
| B4311 | 286 | 0.009 | 0.009 | 0.008 | 0.016 | 0.02 | 1.8 | >1000 | 10 | 12.5 | 8.0 | 73.3 | 8.25 | --- | 1.4 | 0 | 113.0±31.0 |
| B4314 | 283 | 0.011 | 0.012 | 0.073 | 0.037 | 0.03 | 2 | >1000 | >19 | >48 | 7.62 | 84 | 4.61 | --- | 3.46 | Not done | 55.5±7.3 (D ₅₀ 16 µM) |
| B4316 | 294 | 0.025 | 0.011 | 0.068 | 0.03 | 0.04 | 3.8 | >1000 | >19 | 32 | 6.4 | 66 | 13.2 | --- | 1.4 | 0.3 | 85.5±20.0 |
| B4335 | 400 | 0.33 | 0.07 | 15.63 | 6.5 | 1.8 | 156 | >1000 | >19 | >48 | 5.33 | 38 | 3.5 | --- | 1.0 | 0.009 | 98.4±12.2 |
| B4343 | 285 | 0.007 | 0.0085 | 0.31 | 0.045 | 0.02 | 30 | >1000 | 7.5 | 3 | 3.81 | 9.5 | 2.12 | --- | 1.6 | 0.01 | 97.0±10.0 |
| B4349 | 342 | 0.018 | 0.007 | 0.043 | 0.074 | 0.02 | 0.08 | >1000 | 7.3 | >48 | 4.8 | 50.8 | 33 | --- | 2.4 | 0.002 | 90.0±13.0 |
| B4351 | 344 | 0.003 | 0.005 | 0.071 | 0.027 | 0.03 | 5.8 | >1000 | >16 | 12 | 4.8 | 18.1 | 1.32 | --- | 1.2 | Not done | 117.5±29.1 |
| BeG | 241 | 0.04 | 0.1 | 0.2 | 0.076 | 0.01 | 17 | >1000 | >64 | >75 | 4.33 | 27.5 | 1.89 | --- | 1.03 | 0.023 | 82.2±11.0 |
| PaTrin-2 B.4280 | 326 | 0.003 | 0.003 | 0.05 | 0.019 | 0.03 | 0.85 | >1000 | >16 | >48 | 6.0 | 60 | 33 | 8 | 5.5 | Not done | 69.8±10.3 (D ₅₀ 44µM) |

--- = Not Done

TABLE 4

EFFECT OF INACTIVATOR PRETREATMENT ON SENSITISATION
OF VARIOUS HUMAN CANCER CELL LINES TO TEMOZOLOMIDE

| INACTIVATOR | SENSITISATION FACTOR (D_{50} control/ D_{50} 'B') | | | | | | |
|-------------|--|------|---------|-----------------------------|------|-----|------|
| | MCF-7 | PC3 | DU145** | RAJI | | | |
| | Inactivator dose (10 μ M) | | | Inactivator dose (μ M) | | | |
| | | | | 10 | 1.0 | 0.5 | 0.1 |
| B4311 | --- | 5.56 | 3.75 | 73.3 | 8.25 | --- | 1.4 |
| B4314* | --- | 2.0 | 1.71 | 84.0 | 4.61 | --- | 3.46 |
| B4316 | 8.0 | 7.6 | 3.53 | 66 | 13.2 | --- | 1.4 |
| B4349 | 4.8 | 3.6 | 4.0 | 50.8 | 33.0 | --- | 2.4 |
| BeG | 2.94 | 2.88 | 5.45 | 27.5 | 1.89 | --- | 1.03 |
| PaTrin-2 | 3.13 | 4.6 | 4.14 | 60 | 33.0 | 8.0 | 5.5 |

* Toxic to Raji cells at 10 μ M

** Sensitisation factor = D_{60} control/ D_{60} 'B'

--- Not done

TABLE 5

EFFECT OF INACTIVATOR PRETREATMENT ON SENSITISATION
OF VARIOUS HUMAN CANCER CELL LINES TO BCNU

| INACTIVATOR (10 μ M) | SENSITISATION FACTOR (D_{50} control/ D_{50} 'B') | | | | | |
|-----------------------------|--|------|---------|-----------------------------|-----|------|
| | MCF-7 | PC3 | DU145** | RAJI | | |
| | | | | Inactivator dose (μ M) | | |
| | | | | 10 | 1.0 | 0.1 |
| B4311 | --- | 1.47 | 1.56 | 8.0 | --- | --- |
| B4314* | --- | 1.46 | 1.25 | 7.62 | 7.6 | 3.45 |
| B4316 | 1.37 | 1.35 | 3.57 | 6.4 | --- | --- |
| B4349 | 1.85 | 1.63 | 2.78 | 4.8 | --- | --- |
| BeG | 1.94 | 1.41 | 1.79 | 4.33 | --- | --- |
| PaTrin-2 | 1.61 | 2.11 | 2.08 | 6.0 | --- | --- |

* Toxic to Raji cells at 10 μ M

** Sensitisation factor = D_{60} control/ D_{60} 'B'

--- Not done

TABLE 6A

| Test No. | O ⁶ -Substituent | Yield % (based on solvate) | Solvent for recrystn. | M.p. (decomp.) (°C) | Formula | Analysis | | | |
|----------|-----------------------------|----------------------------|-----------------------|---------------------|---|-------------|------|-------|--|
| | | | | | | C | H | N | |
| B.4280 | 4-bromophenyl | 73 | MeOH | 204-205 | C ₁₀ H ₈ BrN ₃ OS | Found 36.7 | 2.45 | 21.46 | |
| | | | | | | Req. 36.82 | 2.47 | 21.47 | |
| B.4281 | 5-chlorophenyl ^a | 39 | MeCN | 155-158 | C ₁₀ H ₈ ClN ₃ OS | Found 41.81 | 2.86 | 24.10 | |
| | | | | | | Req. 42.63 | 2.86 | 24.86 | |
| B.4283 | 5-cyanothenyl ^b | 10 | MeOH | 200 upwards | C ₁₁ H ₈ N ₆ OS·0.5 H ₂ O | Found 47.01 | 2.94 | 28.24 | |
| | | | | | | Req. 46.97 | 3.23 | 29.88 | |
| B.4294 | 5-methylsulphinyphenyl | 32 | MeOH | 200 upwards | C ₁₁ H ₁₁ N ₃ O ₂ S ₂ | Found 42.58 | 3.62 | 22.27 | |
| | | | | | | Req. 42.71 | 3.58 | 22.64 | |
| B.4298 | 4-chlorophenyl | 34 | MeCN | 194-198 | C ₁₀ H ₈ ClN ₃ OS | Found 42.70 | 2.94 | 24.84 | |
| | | | | | | Req. 42.63 | 2.86 | 24.86 | |
| B.4300 | 4-methoxyphenyl | 44 | MeOH | 189-190 | C ₁₁ H ₁₁ N ₃ O ₂ S | Found 47.73 | 4.15 | 25.05 | |
| | | | | | | Req. 47.64 | 4.00 | 25.26 | |
| B.4313 | 5-bromo-3-thienylmethyl | 7.6 | MeCN | 190 upwards | C ₁₀ H ₈ BrN ₃ OS | Found 37.02 | 2.43 | 20.95 | |
| B.4317 | 4-cyanothenyl | 32 | MeOH | 213-216 | C ₁₁ H ₈ N ₆ OS | Req. 36.82 | 2.47 | 21.47 | |
| | | | | | | Found 48.50 | 2.84 | 30.66 | |
| B.4318 | 4,5-dichlorophenyl | 38 | MeOH | 210 upwards | C ₁₀ H ₇ Cl ₂ N ₃ OS·1H ₂ O | Req. 48.52 | 2.96 | 30.87 | |
| | | | | | | Found 35.94 | 2.67 | 20.96 | |
| B.4321 | 2-chloro-4-picolyl | 10 | MeOH | 234 upwards | C ₁₁ H ₉ ClN ₆ O | Req. 35.94 | 2.71 | 20.96 | |
| | | | | | | Found 47.15 | 3.52 | 29.32 | |
| B.4336 | 5-bromofurfuryl | 39 | MeOH | 180 upwards | C ₁₀ H ₈ BrN ₃ O ₂ ·0.25 H ₂ O | Req. 47.75 | 3.29 | 30.37 | |
| | | | | | | Found 38.22 | 2.71 | 21.93 | |
| | | | | | | Req. 38.18 | 2.72 | 22.26 | |

^a 5.6 mmol alcohol per mmol quaternary salt used in synthesis. ^b Dimethylformamide reaction solvent.

O⁶-Substituted guanines (continuation of Table 6a.)

| Compound, Test No. | O ⁶ -Substituted RCH ₂ | Yield % | Solvent for Recrystn. | M.p. (decomp) (C°) | Formula | Molecular Weight | Analysis | | |
|-----------------------|--|------------|--------------------------|--------------------------|--|---------------------|---------------|----------------|----------------|
| | | | | | | | C | H | N |
| B.4282 | 3-picoly N-oxide | 54 | MeOH | 244-254 | C ₁₁ H ₁₀ N ₆ O ₂ | 258 | | | |
| B.4309 | 5-methylsulphonyl- thienyl | 12 | EtOH | 206-209 | C ₁₁ H ₁₁ N ₅ O ₃ S ₂ · ½ C ₂ H ₅ OH | 348 | Found Req. | 41.46 41.37 | 20.13 20.10 |
| B.4319 | 6-chloro-3-picoly | 58 | MeOH | > 215 | C ₁₁ H ₉ ClN ₆ O · ½ H ₂ O | 285.7 | Found Req. | 46.01 46.25 | 29.05 29.42 |
| B.4320 | 5-bromo-3-picoly | 56 | MeOH | > 220 | C ₁₁ H ₉ BrN ₆ O · ½ H ₂ O | 330 | Found Req. | 40.02 39.87 | 25.28 25.28 |
| B.4354 | 4-isothiazolyl | 28 | MeOH | > 200 | C ₉ H ₈ N ₆ OS · ¾ H ₂ O | 261.8 | Found Req. | 41.59 41.32 | 31.53 32.16 |
| B.4356 | 4-methylthiothienyl | 30 | MeOH | | C ₁₁ H ₁₁ N ₅ OS ₂ | 293.4 | | | |
| B.4357 | 5-iodo-3-thienyl- methyl | 23 | MeOH | > 200 | C ₁₀ H ₈ IN ₅ OS | 373 | | | |
| B.4361 | 4-methyl- sulphonylthienyl | 95 | MeOH | 170-172 | C ₁₁ H ₁₁ N ₅ O ₃ S ₂ | 325 | Found Req. | 40.2 40.61 | 21.01 21.53 |
| B.4366 | naphtho[2,1-b]- thiophen-2-yl- methyl | 81 | MeOH | > 150 | C ₁₈ H ₁₃ N ₅ OS | 347 | | | |
| B.4373 | 4-azidothienyl | 37 | MeOH | > 195° | C ₁₀ H ₈ N ₈ SO | 288 | | | |
| B.4377 | 4-methyl- sulphonylthienyl | 55 | MeOH | 204-206 | C ₁₁ H ₁₁ N ₅ O ₂ S ₂ | 309 | | | |
| B.4378 | 5-phenylthienyl | 54 | CH ₃ CN | > 170 | C ₁₆ H ₁₃ N ₅ OS | 323 | | | |

TABLE 6B

| Test No. | O ⁶ -Substituent | λ_{max} (nm)(MeOH) | δ_H [ppm from TMS; (CD ₃) ₂ SO], J (Hz) |
|----------|-----------------------------|---|---|
| B. 4280 | 4-bromothienyl | 238, 284 (RCH ₂ OH:233) | 5.65(s), 6.40(s), 7.37(d), 7.71(d), 7.85(s), 12.49(s). |
| B. 4281 | 5-chlorothienyl | 247, 284 (RCH ₂ OH:245) | 5.59(s), 6.40(s), 7.06(d), 7.22(d), 7.87(s), 12.47(bs). |
| B. 4283 | 5-cyanothienyl | 247, 272 | 5.73(s), 6.46(s), 7.49(d), 7.87(s), 7.92(d), 12.54(bs). |
| B. 4294 | 5-methylsulphinylthienyl | 243, 284(sh) [RCH ₂ OH:240, 274(sh)]. | 2.93(s), 5.73(s), 6.41(s), 7.40(d), 7.52(d), 7.88(s), 12.52(bs). |
| B. 4298 | 4-chlorothienyl | 238, 284 (RCH ₂ OH:240) | 5.64(s), 6.42(s), 7.34(d), 7.62(d), 7.86(s), 12.51(s). |
| B. 4300 | 4-methoxythienyl | 245(sh), 282 (RCH ₂ OH: 258) | 3.75(s), 5.57(s), 6.37(s), 6.60(d), 7.01(d), 7.85(s), 12.48(s). |
| B. 4313 | 5-bromo-3-thienylmethyl | 240, 284 (RCH ₂ OH: 236) | 5.42(s), 6.38(s), 7.40(d), 7.72(d), 7.85(s), 12.47(s). |
| B. 4317 | 4-cyanothienyl | 244, 284 (RCH ₂ OH: 244) | 5.68(s), 6.44(s), 7.74(d), 7.86(s), 8.60(d), 12.50(s). |
| B. 4318 | 4,5-dichlorothienyl | 243, 285 (RCH ₂ OH:243) | 5.58(s), 6.45(s), 7.41(s), 7.87(s), 12.52(s). |
| B. 4321 | 2-chloro-4-picoyl | 241, 272(sh), 285 [RCH ₂ OH: 262, 268(sh)]. | 5.58(s), 6.36(s), 7.51(bs), 7.61(bs), 7.91(bs), 8.44(bs), 12.56(bs). |
| B. 4336 | 5-bromofurfuryl | 220, 284 (RCH ₂ OH: 223) | 5.42(s), 6.39(s), 6.64(d), 6.78(d), 7.85(s), 12.49(s). |

06-Substituted guanines (continuation of Table 6B)

| Compound Type, Test No. | O ⁶ -Substituent RCH ₂ | λ_{max} (nm) | δ_H [ppm from TMS, (CD ₃) ₂ SO- <i>d</i> ₆] (Hz) |
|-------------------------|---|--------------------------|--|
| B.4282 | 3-picoly <i>N</i> -oxide | 271 | 5.48(s), 6.41(s), 7.47(m), 7.87(s), 8.22(m), 8.42(s), 12.52(s). |
| B.4309 | 5-methylsulphonyl- thienyl | 242, 284 | 5.75(s), 6.43(s), 7.47(d), 7.74(d), 7.87(s), 12.52(s). |
| B.4319 | 6-chloro-3-picoly | 242, 276 | 5.53(s), 6.38(s), 7.59(d), 7.87(s), 8.05(dd), 8.64(d), 12.48(s). |
| B.4320 | 5-bromo-3-picoly | 242, 281 | 5.53(s), 6.41(s), 7.86(s), 7.86(s), 8.26(dd), 8.73(d), 8.78(d), 12.50(s). |
| B.4354 | 4-isothiazolyl | 244, 284 | 5.58(s), 6.41(s), 7.84(s), 8.81(s), 9.22(s), 12.47(s). |
| B.4356 | 4-methylthio-thenyl | 236, 283 | 2.48(s), 5.62(s), 6.40(s), 7.26(m), 7.85(s), 12.48(s). |
| B.4357 | 5-iodo-3- thienylmethyl | 240, 283 | 5.43(s), 6.38(s), 7.48(d), 7.77(s), 7.84(s), 12.47(s). |
| B.4361 | 4-methylsulphonyl- thienyl | 240, 285 | 3.26(s), 5.70(s), 6.40(s), 7.72(s), 7.85(s), 8.38(d), 12.49(s). |
| B.4366 | naphtho[2,1- <i>b</i>]- thiophen-2-ylmethyl | 244, 286sh 295, 306sh | 5.90(s), 6.47(s), 7.60(t), 7.69(t), 7.86(t), 8.04(dd), 8.44(s), 8.51(d), 12.51(s). |
| B.4373 | 4-azidothienyl | 227, 280 | 5.64(s), 6.36(s), 7.20(s), 7.28(s), 7.84(s), 12.47(s). |
| B.4377 | 4-methylsulphonyl- thienyl | 241, 285 | 2.82(s), 5.68(s), 6.33(s), 7.60(s), 7.82(s), 8.01(s), 12.45(s). |
| B.4378 | 5-phenylthienyl | 244sh, 289 | 5.67(s), 6.32(s), 7.31(m), 7.41(m), 7.41(m), 7.63(d), 7.82(s), 12.43(s). |

TABLE 7INACTIVATOR

| | M.Wt | I ₅₀ (μM) hAT | Raji I ₅₀ (μM) | Stability T 1/2(h) By Spec |
|--|------|-----------------------------|------------------------------|-------------------------------|
|--|------|-----------------------------|------------------------------|-------------------------------|

B.4280

Q⁶-(4-bromothienyl)guanine

326 0.0034

B.4281

Q⁶-(5-chlorothienyl)guanine

281.7 0.004 >10

B.4282

Q⁶-(oxido-3-picolyl)guanine

276 1.4 >20

B.4283

Q⁶-(5-cyanothienyl)guanine

272 0.005 >20

B.4294

Q⁶-(5-methylsulphinythienyl)guanine

309 0.03 >10

B.4298

Q⁶-(4-chlorothienyl)guanine

282 0.008 0.005 >16

B.4300

Q⁶-(4-methoxythienyl)guanine

277 0.0165 0.83

TABLE 7 (continued)

| <u>INACTIVATOR</u> | M.Wt | I ₅₀ (μM) hAT | Raji I ₅₀ (μM) | Stability T 1/2 (h) By Spec |
|---|------|-----------------------------|------------------------------|--------------------------------|
| B.4309 Q ⁶ -(5-methylsulphonylthienyl)guanine | 325 | 0.072 | | >16 |
| B.4313 Q ⁶ -(5-bromo-3-thienylmethyl)guanine | 326 | 0.0065 | 0.035 | |
| B.4317 Q ⁶ -(4-cyanothienyl)guanine | 272 | 0.0028 | | >19 |
| B.4318 Q ⁶ -(4,5-dichloroethenyl)guanine | 348 | 0.015 | | 2.5 |
| B.4319 Q ⁶ -(6-chloro-3-picolyl)guanine | 277 | 0.2 | | >13 |
| B.4320 Q ⁶ -(5-bromo-3-picolyl)guanine | 321 | 0.25 | | >13 |
| B.4321 Q ⁶ -(2-chloro-4-picolyl)guanine | 277 | 0.04 | | >16 |

TABLE 7 (continued)

INACTIVATOR

| | M.Wt | I ₅₀ (μ M) hAT | Raji I ₅₀ (μ M) | Stability T 1/2 (h) By Spec |
|--|------|-----------------------------------|------------------------------------|--------------------------------|
| B.4336 Q ⁶ -(5-bromofurfuryl)guanine | 310 | 0.02 | | 0.32 |
| B.4354 Q ⁶ -(4-isothiazolylmethyl)guanine | 248 | 0.07 | | |
| B.4356 Q ⁶ -(4-methylthiohenyl)guanine | 293 | 0.0095 | | |
| B.4357 Q ⁶ -(5-iodo-3-thienylmethyl)guanine | 447 | 0.009 | | >16 |
| B.4361 Q ⁶ -(4-methylsulphonylphenyl)guanine | 325 | 0.2 | | >16 |
| B.4366 Q ⁶ -(naphtho[2,1-b]thiophen-2-ylmethyl)guanine | 347 | 0.05 | | |

TABLE 7 (continued)

INACTIVATOR

| | M.Wt | I ₅₀ (μM) hAT | Raji I ₅₀ (μM) | Stability T 1/2 (h) By Spec |
|---|------|-----------------------------|------------------------------|--------------------------------|
| B.4368 9-(B-D-arabinofuranosyl)-Q ⁶ -(4-bromophenyl)guanine | 458 | 0.115 | | |
| B.4369 Q ⁶ -(4-bromophenyl)-9-(ethoxymethyl)guanine | 384 | 0.28 | | |
| B.4370 Q ⁶ -(4-bromophenyl)-9-(octyloxymethyl)guanine | 468 | 1.2 | | |
| B.4373 Q ⁶ -(4-azidothienyl)guanine | 288 | 0.0063 | | |
| B.4377 Q ⁶ -(4-methylsulphonylthienyl)guanine | 309 | 0.15 | | |
| B.4378 Q ⁶ (5-phenylthienyl)guanine | 323 | 0.75 | | |
| B.4379 Q ⁶ -(4-bromophenyl)-2-deoxyguanosine | 442 | 0.095 | | |

TABLE 7B

INACTIVATOR

| M.Wt | In vitro I ₅₀ (μM) | | | Raji I ₅₀ | Stability T 1/2 (h) | |
|---|-------------------------------|------|------|----------------------|---------------------|------------------|
| | hAT | mAT | rAT | chAT | ogt | By Spec By Assay |
| B.4363 | | | | | | |
| Q ⁶ -(4-bromothienyl)guanosine | 458 | 0.08 | 0.24 | 0.95 | 30 | >16 >48 |

Blank space = not done

TABLE 8

ATASE ACTIVITY IN VARIOUS TISSUES OF NU/NU MICE AFTER
TREATMENT WITH 10mg/kg (IP) B.4280
MEAN ACTIVITY (fm/mg)

| <u>Tissue</u> | <u>24h</u> | <u>48h</u> | <u>Control*</u> |
|----------------------|--------------|--------------|-----------------|
| Tumour | 36 ± 7.79 | 140 ± 43.87 | 125 |
| Liver | 89.7 ± 10.14 | 100.7 ± 8.73 | 110** |
| Lung | 15.3 ± 2.05 | 24 ± 2.83 | 43 |
| Kidney | 24.3 ± 4.03 | 28.7 ± 4.11 | 33 |
| Spleen | 41 ± 5.35 | 68.3 ± 9.53 | 81 |
| Brain | 13.7 ± 2.05 | 16.3 ± 1.25 | 14 |
| Testis | 45 ± 7.48 | 44 ± 1.41 | 45 |
| Bone Marrow (pooled) | 42 | 61 | 30 |

* control values taken from a separate experiment

** mean of 2 control liver values

Table 8.
 Effect of B.4280 on ATase activity in several tissues of nude mice. Animals were given a single dose of B.4280 (10mg/kg i.p.) and sacrificed 24 or 48 hours later.

TABLE 9TOXICITY OF INACTIVATORS IN COMBINATION WITH BCNU IN DBA₂ MICE

| <u>INACTIVATOR</u> (60mg/kg) | <u>%SURVIVAL AFTER 14 DAYS</u> | | |
|---------------------------------|--------------------------------|--------------|--------------|
| | 20mg/kg BCNU | 16mg/kg BCNU | 12mg/kg BCNU |
| 06-benzylguanine | 33 (2/6) | 0 (0/6)* | 50 (3/6)** |
| B.4205 | 0 (0/6) | 50 (3/6)* | 100 (6/6)** |
| B.4280 | 93 (14/15) | 100 (15/15) | 100 (15/15) |

* 15mg/kg BCNU

** 10mg/kg BCNU

All agents were given as a single i.p. dose

Table 9
Effect of ATase inactivators on the acute toxicity of bis-chloroethylnitrosourea (BCNU) in DBA₂ mice.

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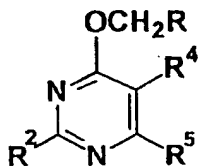
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CLAIMS

1. A 6-hetarylalkyloxy pyrimidine derivative of formula II



II

wherein

R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N, or S, or a substituted derivative thereof; or (iii) phenyl or a substituted derivative thereof,

R² is selected from H, C₁-C₅ alkyl, halogen or NH₂,

R⁴ and R⁵ which are the same or different are selected from H, NH-Y' or NO_n

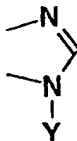
wherein

Y' is H, ribosyl, deoxyribosyl, arabinosyl, R''XCHR''' wherein X is O or S, R'' is alkyl and R''' is H or alkyl, or substituted derivatives thereof,

n = 1 or 2

or R⁴ and R⁵ together with the pyrimidine ring form a 5 or 6-membered ring structure containing one or more hetero atoms, and pharmaceutically acceptable salts thereof,

with the proviso that R² is not NH₂ if R⁴ and R⁵ form a ring structure IX



IX

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wherein Y is H, ribosyl, deoxyribosyl, or
 $R''XCHR'''$ wherein X is O or S, R'' and R''' are alkyl, or
 substituted derivatives thereof,

5

and with the proviso that R is not phenyl in the following
 circumstances a) to h):

a) if R^2 and R^5 are NH_2 and R^4 is NO or NO_2

10

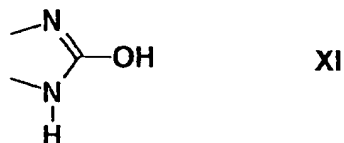
b) if R^2 is NH_2 and R^4 and R^5 form a ring
 structure X



15

c) if R^2 is NH_2 and R^4 and R^5 form a ring
 structure XI

20



25

d) if R^2 is NH_2 , R^4 is NO_2
 and R^5 is H or CH_3

e) if R^2 , R^4 and R^5 are NH_2 .

f) if R^2 and R^5 are NH_2 and R^4 is H

30

g) if R^2 is H, R^4 is NO_2 and R^5 is NH_2

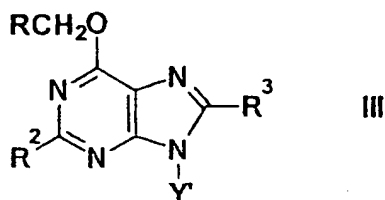
h) if R^2 is F or OH, and R^4 and R^5 form a ring structure XII

35



2. A compound according to claim 1 which is of Formula III

5



10 wherein:

R is as defined in claim 1

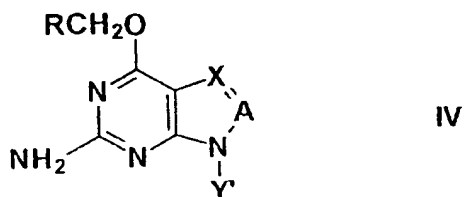
Y' is as defined in claim 1;

R² is H, NH₂, C₁-C₅ alkyl or halogen;

15 R³ is H or OH;

3. A compound according to claim 1 which is of Formula IV

20



wherein:

25

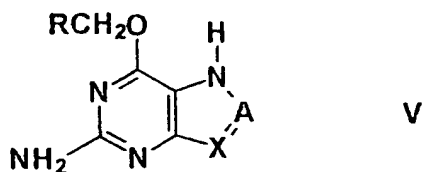
R and Y' are as defined in claim 1;

X is CH or N;

A is CH or N;

4. A compound according to claim 1 which is of Formula V

30



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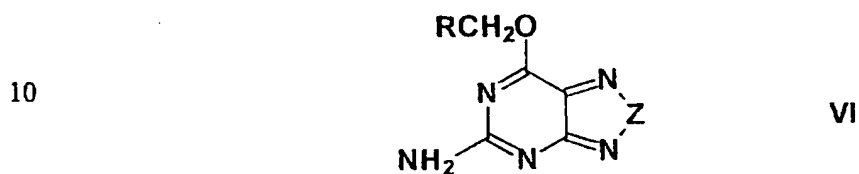
wherein:

R is as defined in claim 1

X is CH or N

5 A is CH or N.

5. A compound according to claim 1 which is of Formula VI



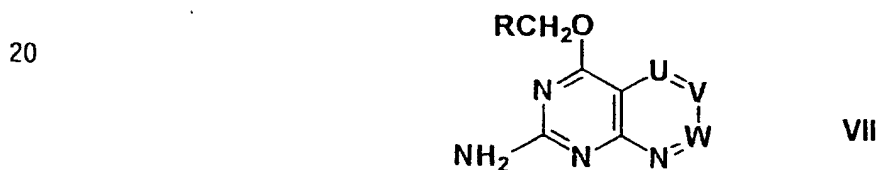
wherein:

R is as defined in claim 1;

15

Z is O or S or CH = CH.

6. A compound according to claim 1 which is of Formula VII



wherein:

25 R is as defined in claim 1;

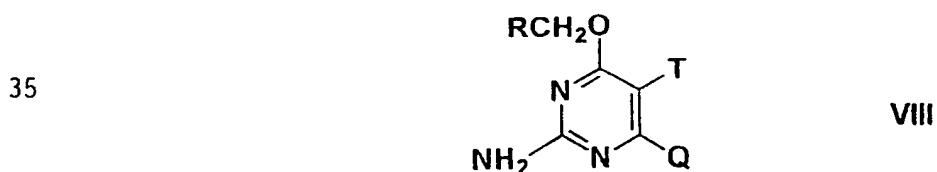
U is CH or N;

V is CH or N;

W is CH or N;

30 provided that U, V and W are not all CH.

7. A compound according to claim 1 which is of Formula VIII



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wherein:

R is as defined in claim 1

T is H, NH_2 or NO_n where $n = 1$ or 2 ;

5 Q is H, NH_2 or NO_n where $n = 1$ or 2 .

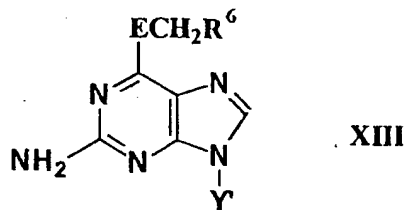
8. A compound according to claim 1, 2 or 3 wherein Y' is H, ribosyl, deoxyribosyl, arabinosyl, $\text{HOCH}_2\text{CH}_2\text{OCH}_2-$ or $\text{R}''\text{XCHR}'''$ wherein X is O or S, R'' and R''' are alkyl, or
10 substituted derivatives thereof.
9. A compound according to claim 1 wherein R is a 5-membered heterocyclic ring, having at least one S atom therein.
- 15 10. A compound according to claim 1 wherein R is selected from a thiophene ring, a furan ring, and substituted derivatives thereof.
11. A compound according to claim 1 wherein R includes a heterocyclic and/or carbocyclic ring substituted by halo, haloalkyl, cyano, SO_nR^8 where R^7 is alkyl and
20 $n = 0, 1$ or 2 , or $-\text{COOR}^8$ wherein R^8 is alkyl.
12. A compound according to claim 1 wherein R is selected from a thiophene ring, a furan ring and substituted derivatives thereof
25 selected from bromo- and cyano-substituted derivatives thereof.
13. A compound according to claim 1 wherein R is selected from thiophene and furan rings with a chloro-, bromo- or cyano-substituent in a 1,3- or 1,4-relationship with the
30 methyleneoxy group attached to the pyrimidine residue.
14. A compound according to claim 1 wherein Y' is alkoxymethyl optionally substituted with OH on the alkyl of the alkoxy group.
- 35 15. A compound according to claim 1 which is selected from

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- \underline{Q}^6 -(4-bromophenyl)-8-thiaguanine.
 \underline{Q}^4 -(4-chlorophenyl)pterin.
 \underline{Q}^6 -piperonyl -7-deaza-8-azaguanine.
 5 \underline{Q}^6 -(4-bromophenyl)-8-hydroxyguanine.
 \underline{Q}^6 -(4-chlorophenyl)-2, 4-diamino-6-hydroxy-5-nitrosopyrimidine.

16. Guanine derivatives of formula XIII

10



wherein

15

E is O or S,

Y' is as defined in claim 1,

R⁶ is a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hereto atom chosen from O, N or S, or a substituted derivative thereof,

20

and pharmaceutically acceptable salts thereof, with the proviso that compounds published in WO 94/29312 are disclaimed.

25

17. A compound according to claim 16 wherein R⁶ is a 5-membered heterocyclic ring, having at least one S atom therein.

18. A compound according to claim 16 wherein R⁶ is selected from a thiophene ring, a furan ring, and substituted derivatives thereof.

30

19. A compound according to claim 16 wherein R⁶ includes a heterocyclic and/or carbocyclic ring substituted by halo, haloalkyl, cyano, SO_nR⁷ where R⁷ is alkyl and n = 0, 1 or 2, or -COOR⁸ wherein R⁸ is alkyl.

35

20. A compound according to claim 16 wherein R⁶ is selected from a thiophene ring, a furan ring and substituted derivatives thereof

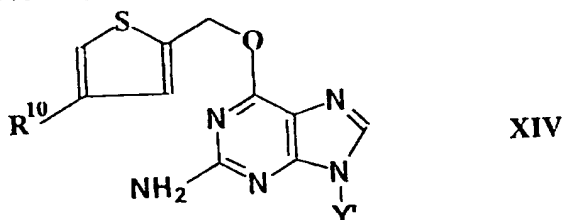
- 85 -

selected from bromo- and cyano-substituted derivatives thereof.

21. A compound according to claim 16 wherein R^6 is selected from
 5 thiophene and furan rings with a chloro-, bromo- or
 cyano-substituent in a 1,3- or 1,4-relationship with the
 methyleneoxy group attached to the pyrimidine residue.

22. Guanine derivatives of formula XIV:

10



- 15 wherein R^{10} is bromo, chloro or cyano, and Y' is as defined in
 claim 1.

23. A compound according to claim 22 wherein Y' is H, ribosyl,
 deoxyribosyl, or $R''XCHR'''$ wherein X is O or S, R'' is alkyl and
 20 R''' is H or alkyl, or substituted derivatives thereof.

24. \underline{Q}^6 -(4-bromophenyl)guanine.

25. A compound according to claim 22 which is
 \underline{Q}^6 -(4-bromophenyl)-9-(2-hydroxyethoxymethyl)guanine.

26. A compound according to claim 22 which is
 9(β-D-arabinofuranosyl)- \underline{Q}^6 -(4-bromophenyl)guanine.

- 30 27. A compound according to claim 22 which is
 \underline{Q}^6 -(4-bromophenyl)guanosine.

28. A compound according to claim 22 which is
 \underline{Q}^6 -(4-bromophenyl)-2-deoxyguanosine.

35

29. A compound according to claim 20 which is selected from:

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Q⁶-(5-chlorothenyl)guanine

Q⁶-(5-cyanothenyl)guanine

5

Q⁶-(5-methylsulphinythienyl)guanine

Q⁶-(4-chlorothenyl)guanine

10 Q⁶-(4-methoxythenyl)guanine

Q⁶-(5-bromo-3-thienylmethyl)guanine

Q⁶-(4-cyanothenyl)guanine

15

Q⁶-(4,5-dichlorothenyl)guanine

30. A compound according to claim 20 which is selected from

20 Q⁶-(4-methylthiothenyl)guanine

Q⁶-(4-azidothenyl)guanine.

31. A compound according to claim 16 which is selected from

25

Q⁶-(2-chloro-4-picolyl)guanine

Q⁶-(5-bromofurfuryl)guanine

30 32. A pharmaceutical composition comprising a compound according to any of claims 1 to 31 and a pharmaceutically acceptable excipient.

33. A pharmaceutical composition according to claim 32 further comprising an alkylating agent.

35

34. A composition according to claim 33 wherein the alkylating agent is selected from 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU)

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and temozolomide.

35. A method for depleting O^6 -alkylguanine-DNA alkyltransferase
5 activity in a host comprising:

administering to the host an effective O^6 -alkylguanine-DNA
alkyltransferase activity depleting amount of a composition
comprising a compound according to any of claims 1 to 31.

10

36. A method for treating tumour cells in a host comprising:

administering to the host a composition comprising an
inactivator compound according to any of claims 1 to 31 in an amount
15 effective to deplete O^6 -alkylguanine -DNA alkyltransferase
activity sufficiently to enhance the effectiveness of a
chemotherapeutic alkylating agent; and

administering to the host a composition comprising an
20 alkylating agent in an amount which is cytotoxically effective in
combination with the said inactivator compound.

37. Use of a compound according to any of claims 1 to 31 in the
manufacture of a medicament for depleting O^6 -alkylguanine-DNA
25 alkyltransferase activity in tumour cells.

38. A pharmaceutical composition comprising
 O^6 -(4-bromophenyl)guanine and a pharmaceutically acceptable
excipient.

30

39. A pharmaceutical composition according to claim 38 which is
suitable for oral administration.

40. A pharmaceutical composition according to claim 38 further
35 comprising an alkylating agent.

41. A pharmaceutical composition according to claim 40 wherein the

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alkylating agent is selected from 1,3
bis(2-chloroethyl)-1-nitrosourea (BCNU) and temozolomide.

- 5 42. A method for depleting Q^6 -alkylguanine
-DNA alkyltransferase activity in a host comprising:

administering to the host an effective Q^6 -alkylguanine-DNA
alkyltransferase activity depleting amount of a composition
10 comprising Q^6 -(4-bromothienyl)guanine.

43. A method for treating tumour cells in a host comprising:

administering to the host a composition comprising
15 Q^6 -(4-bromothienyl)guanine in an amount effective to deplete
 Q^6 -alkylguanine-DNA alkyltransferase activity sufficiently to
enhance the effectiveness of a chemotherapeutic alkylating agent; and

administering to the host a composition comprising an
20 alkylating agent in an amount which is cytotoxically effective in
combination with Q^6 -(4-bromothienyl)guanine.

44. Q^6 -(methylene[^3H])-(4-bromothienyl)guanine.

25

30

35

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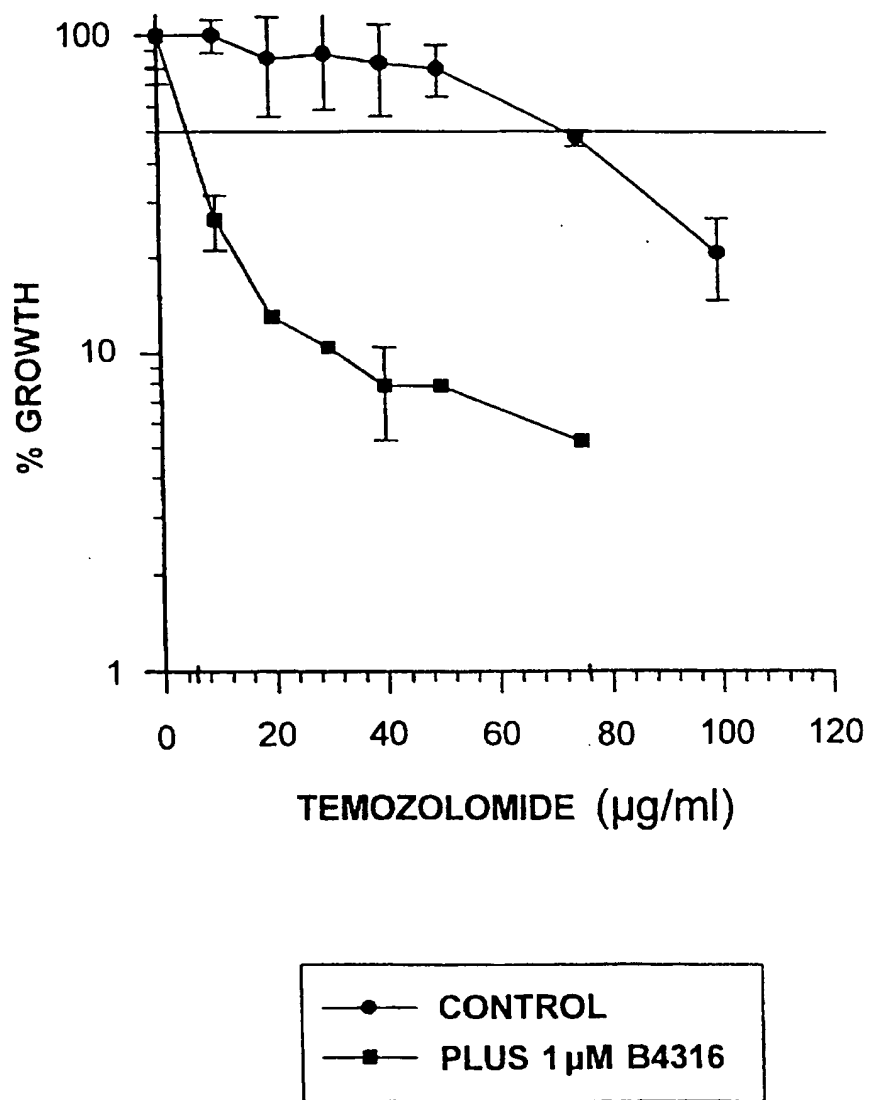


FIG. 1

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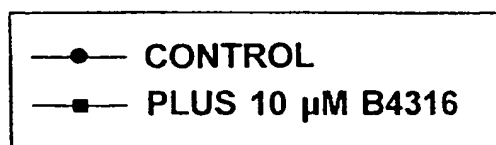
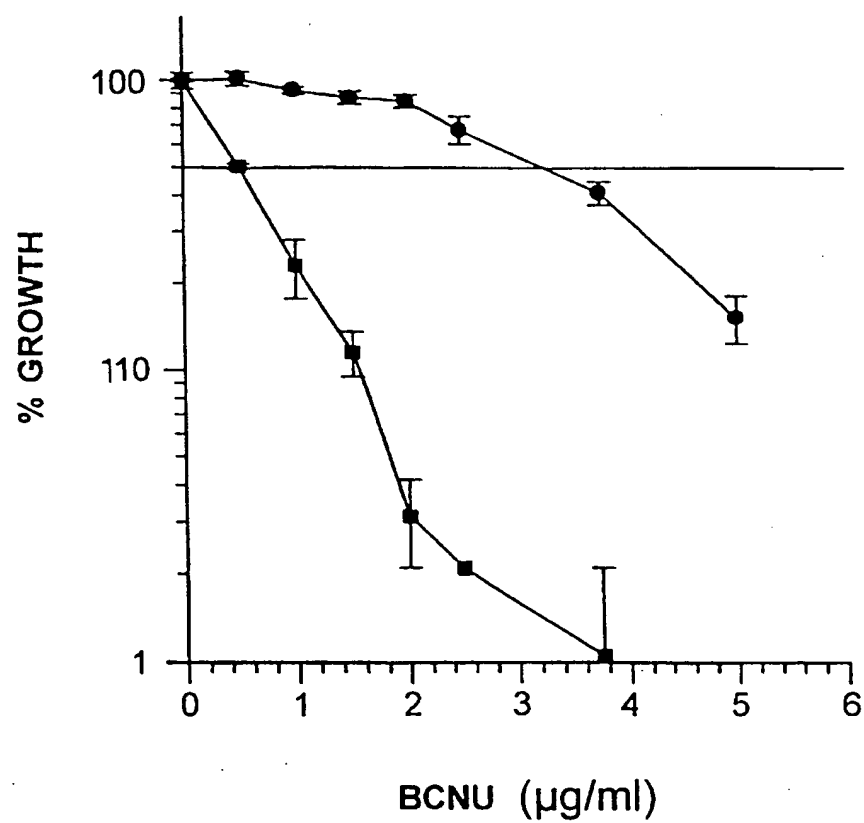


FIG. 2

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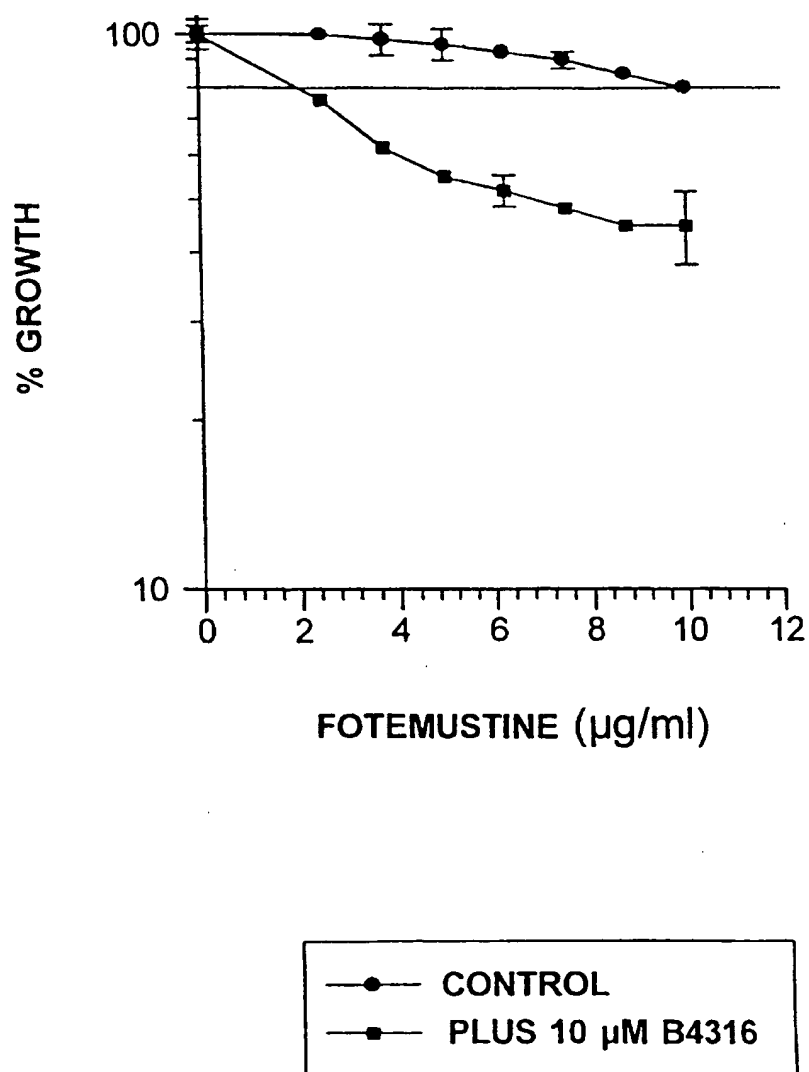


FIG. 3

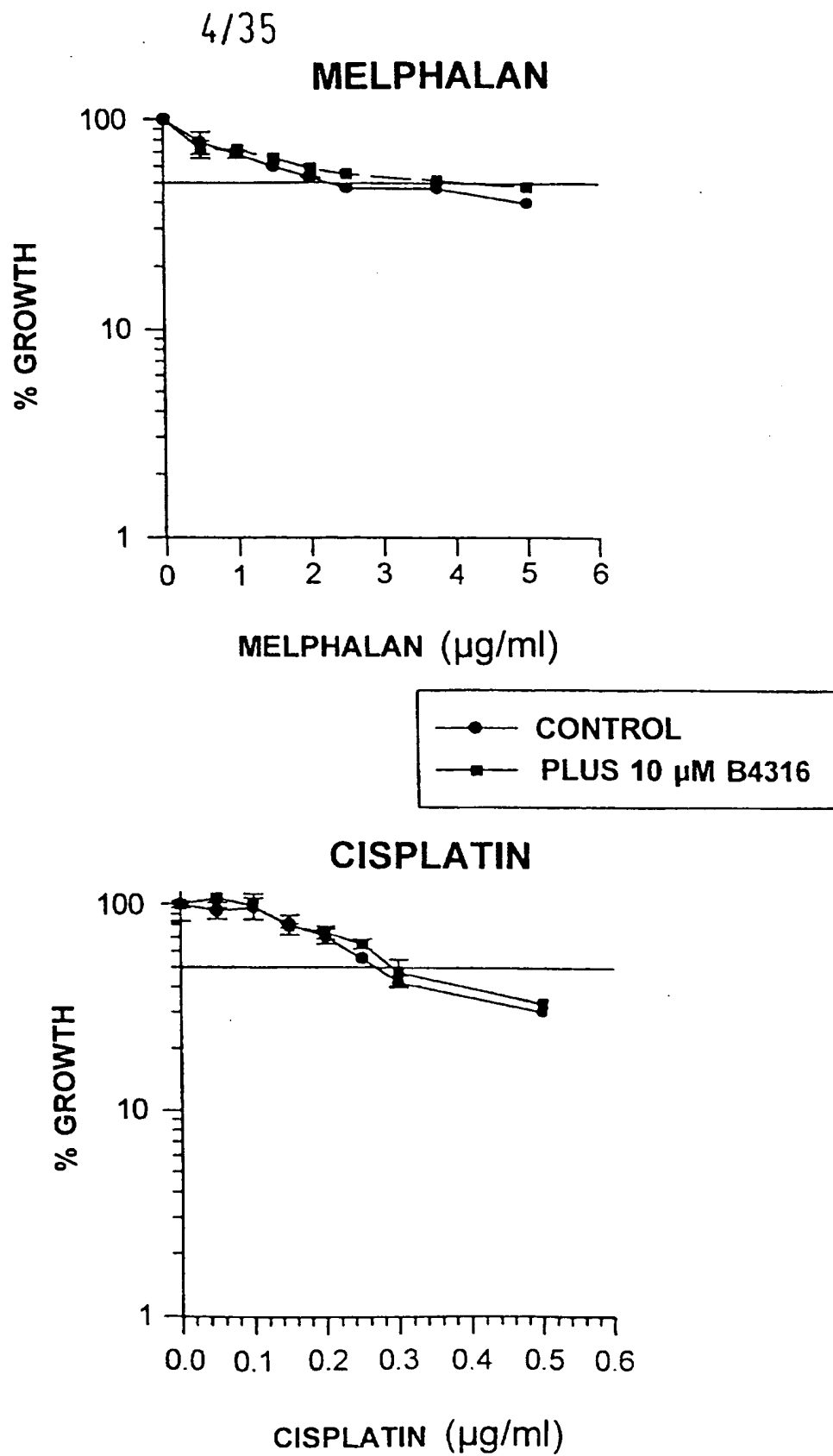


FIG. 4

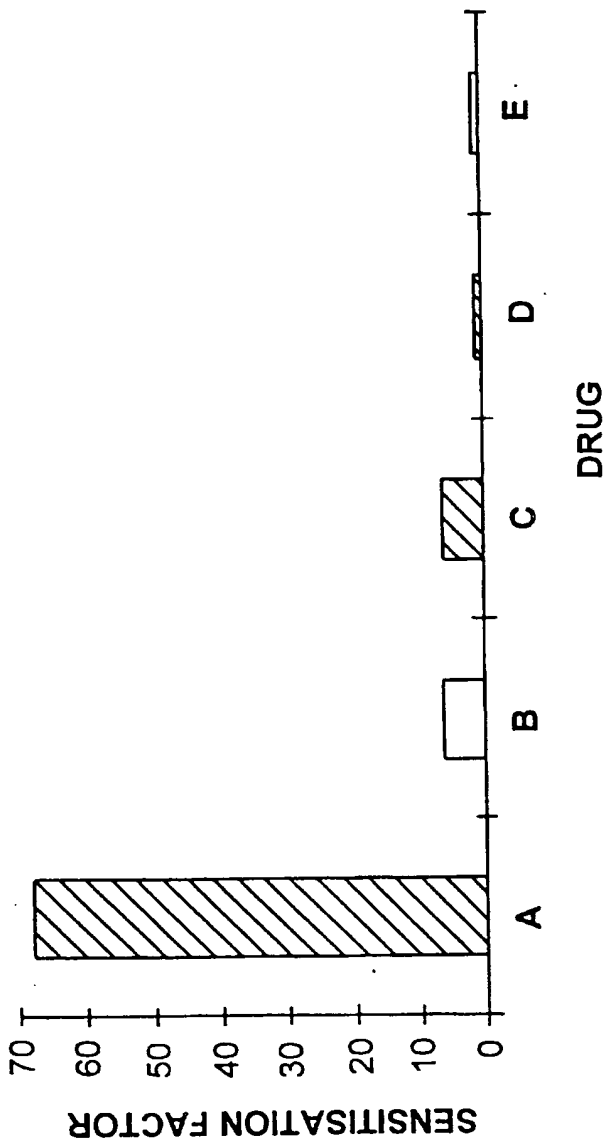


FIG. 5

| DRUG | S.F. |
|------|------|
| A | 67.7 |
| B | 6.4 |
| C | 6.25 |
| D | 1 |
| E | 1 |

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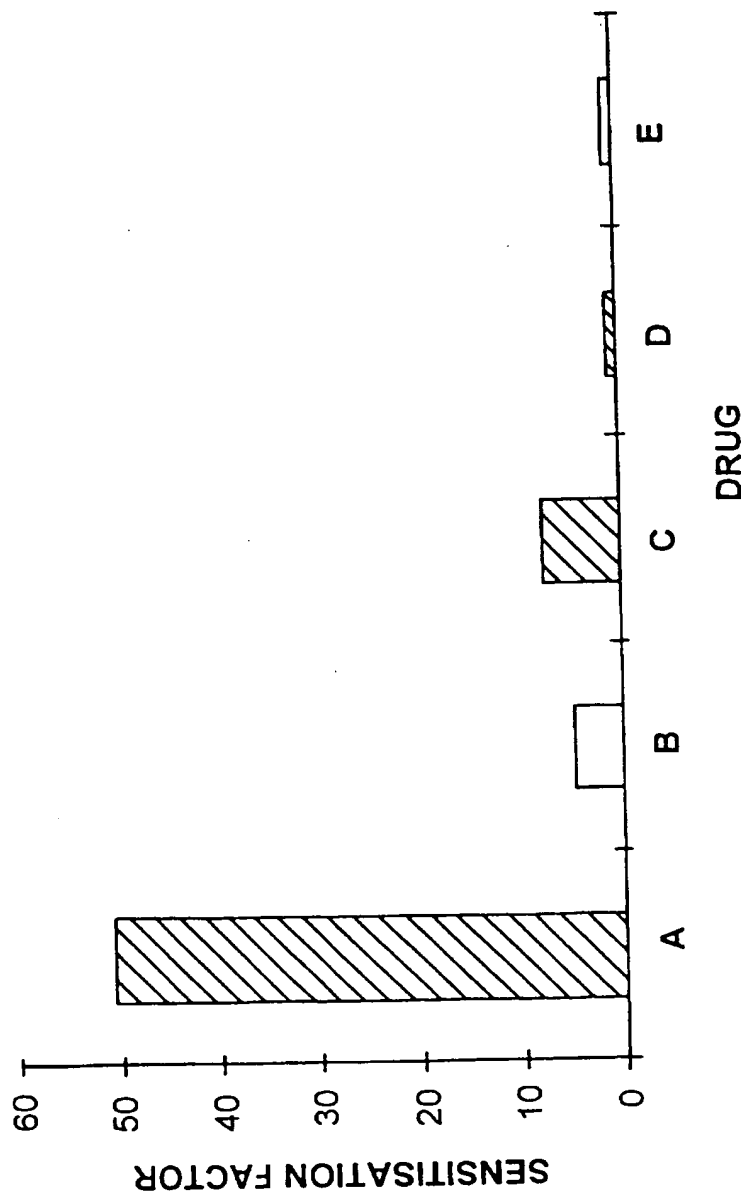


FIG. 6

| DRUG | S.F. |
|----------------|------|
| A TEMOZOLOMIDE | 50.7 |
| B BCNU | 4.8 |
| C FOTEMUSTINE | 7.7 |
| D MELPHALAN | 1 |
| E CISPLATIN | 1 |

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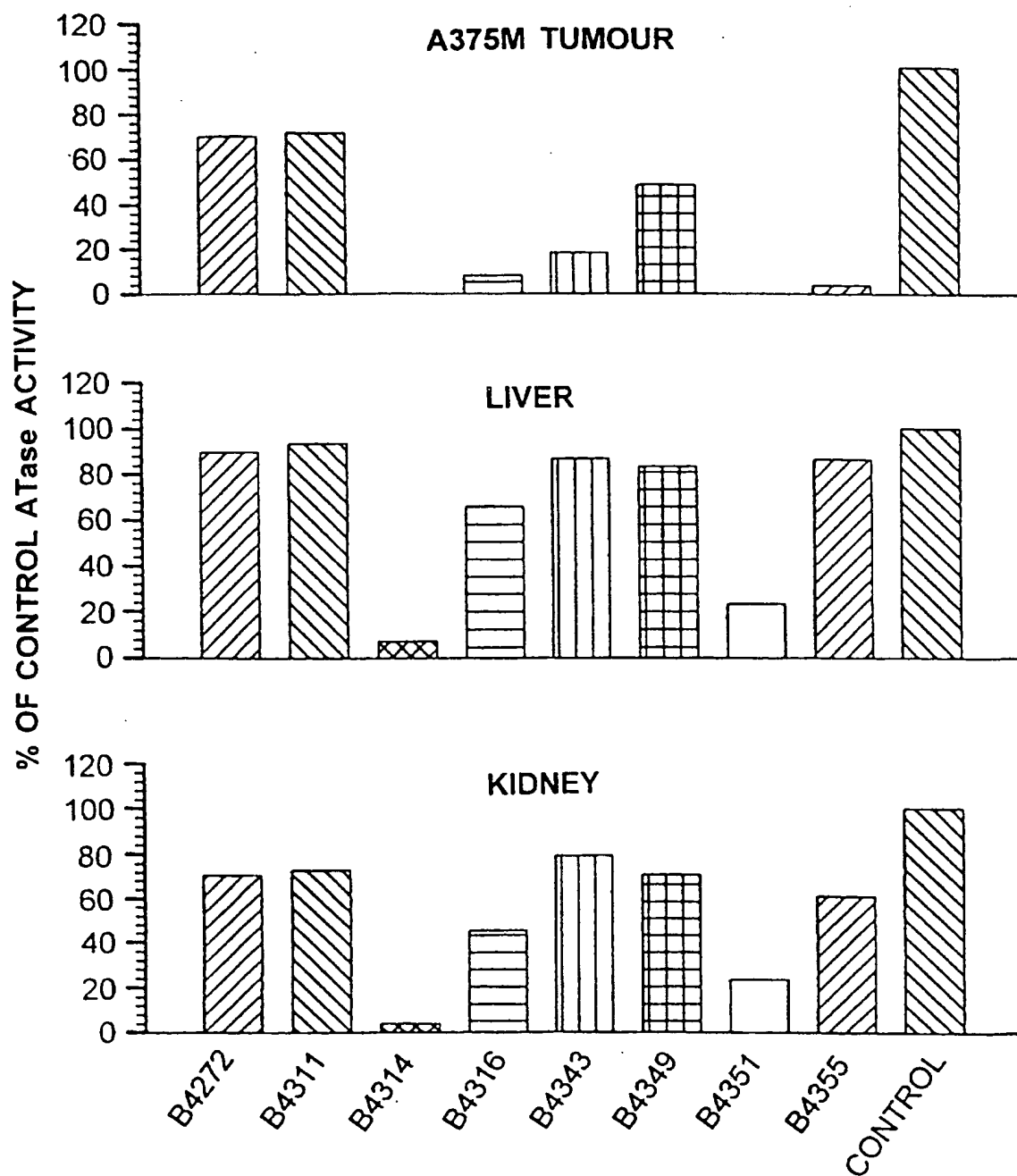
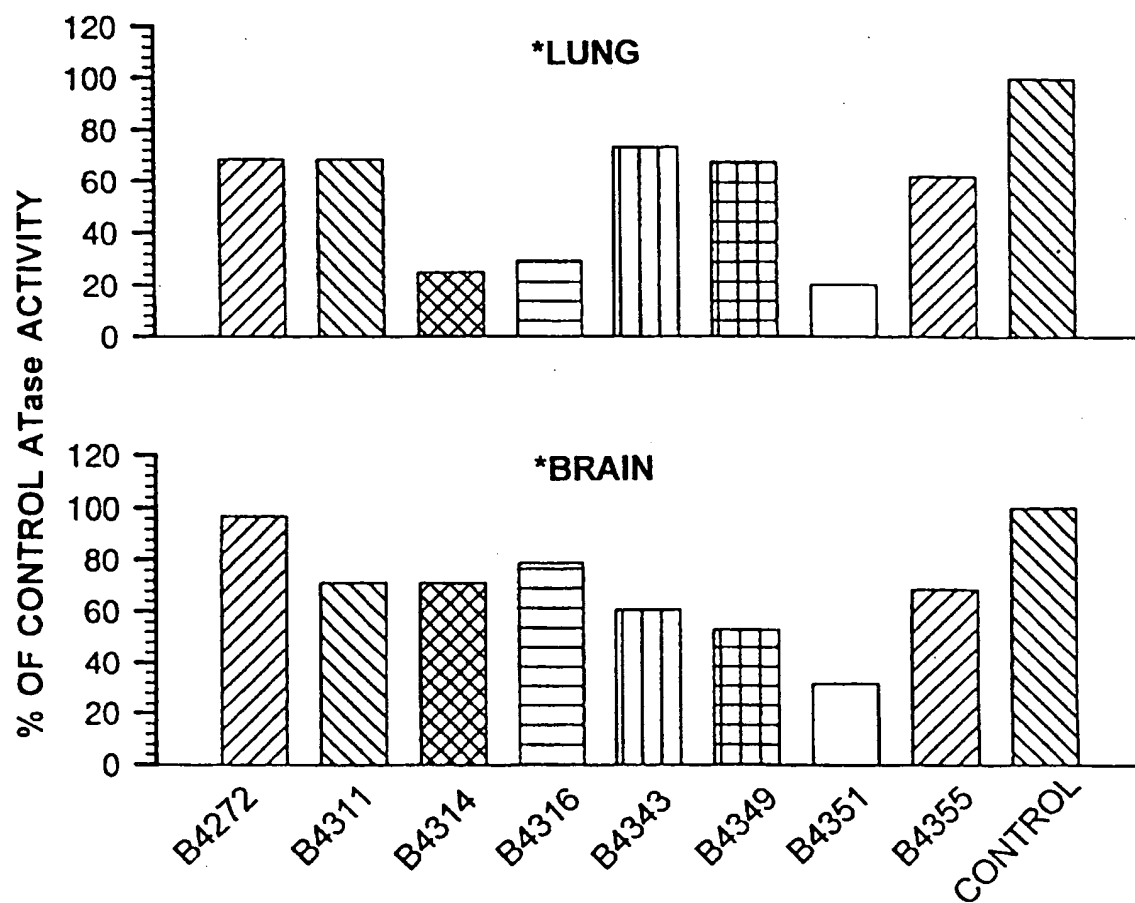


FIG. 7A

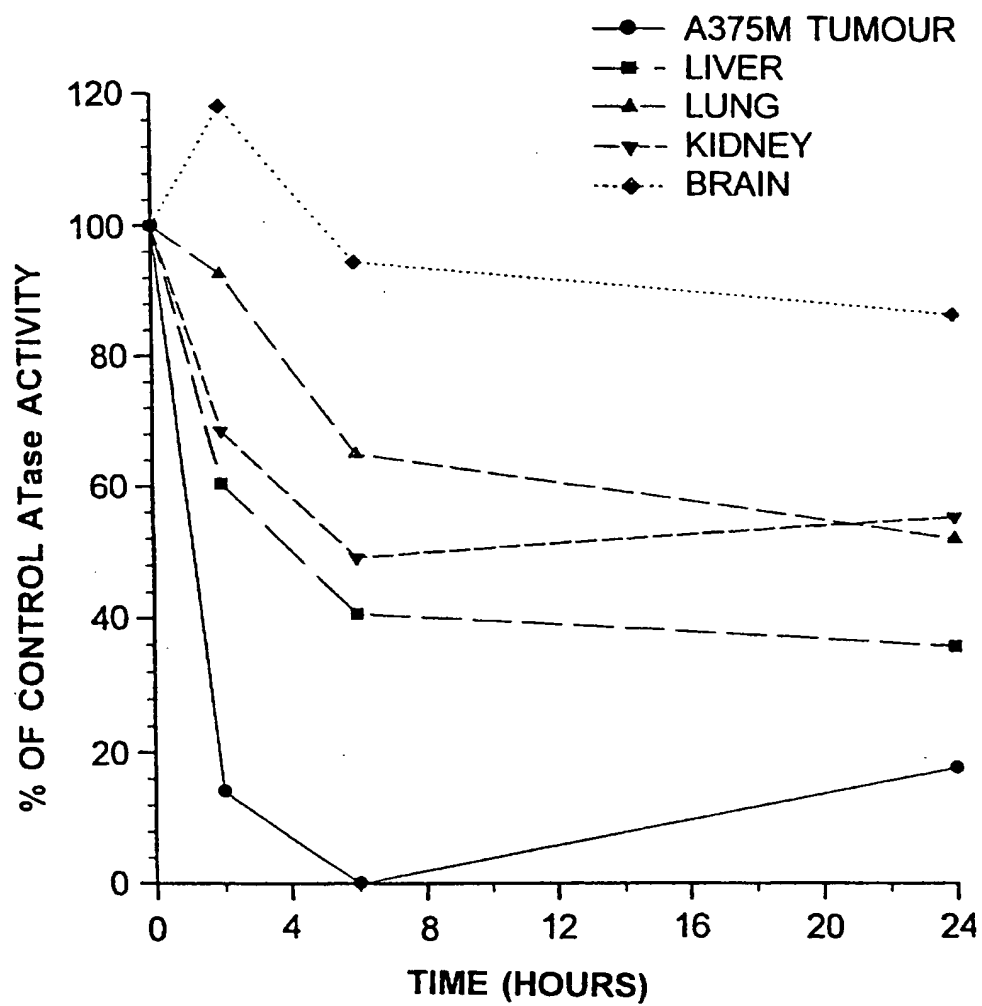
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*HISTORICAL CONTROLS USED

FIG. 7B

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**FIG. 8**

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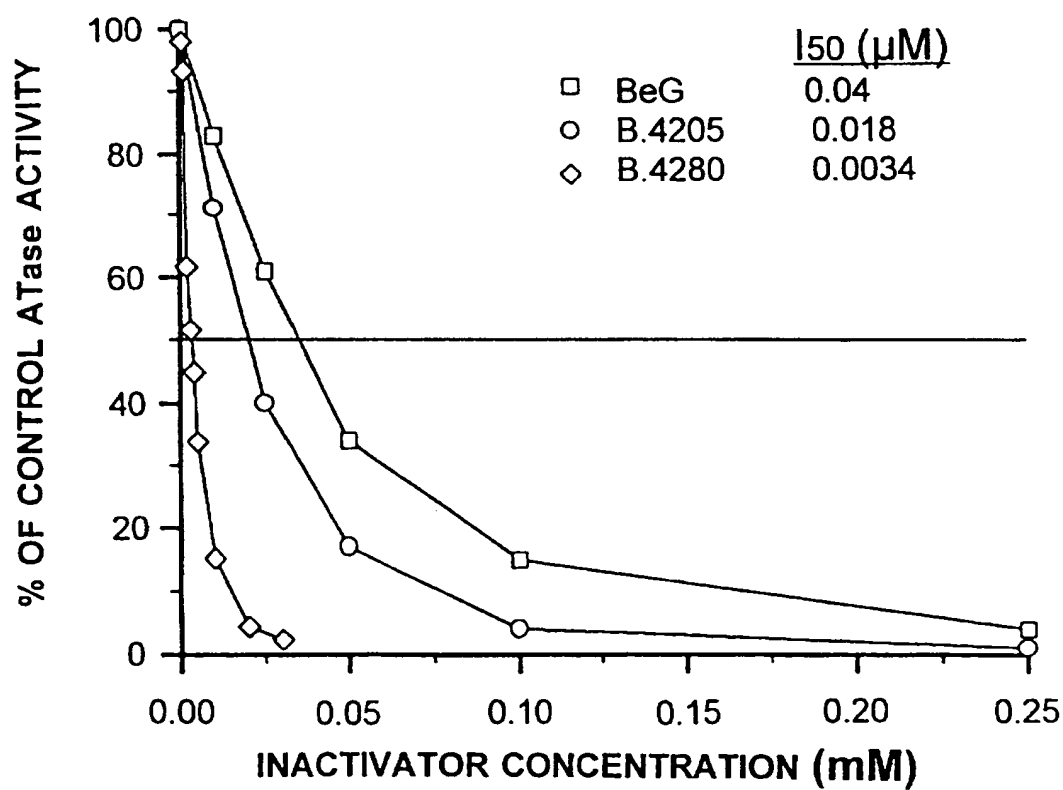
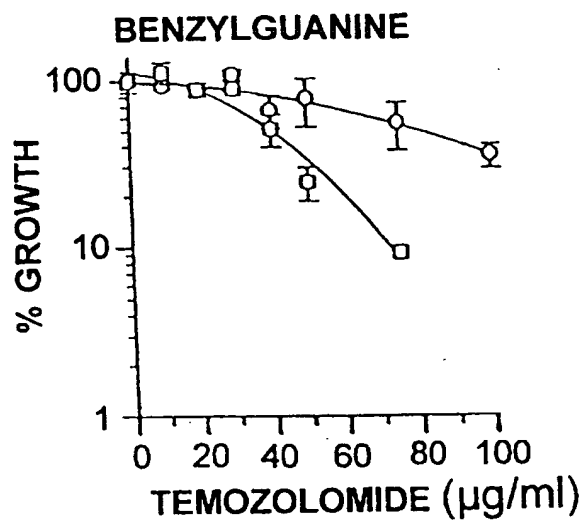


FIG. 9

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○ CONTROL

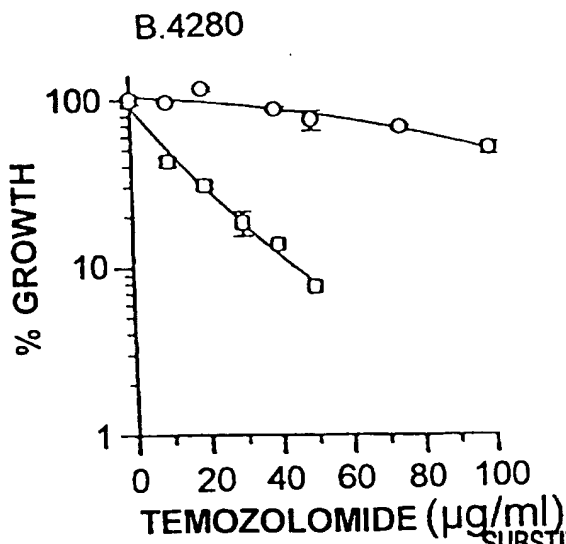
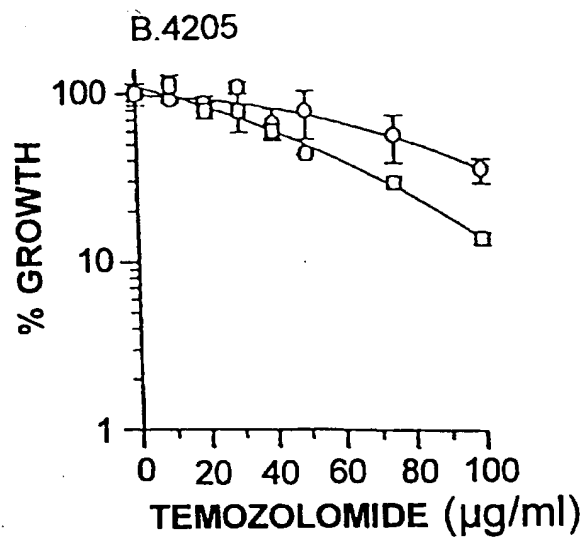
□ 0.5 μM INACTIVATOR

FIG. 10A

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SENSITISATION FACTORS

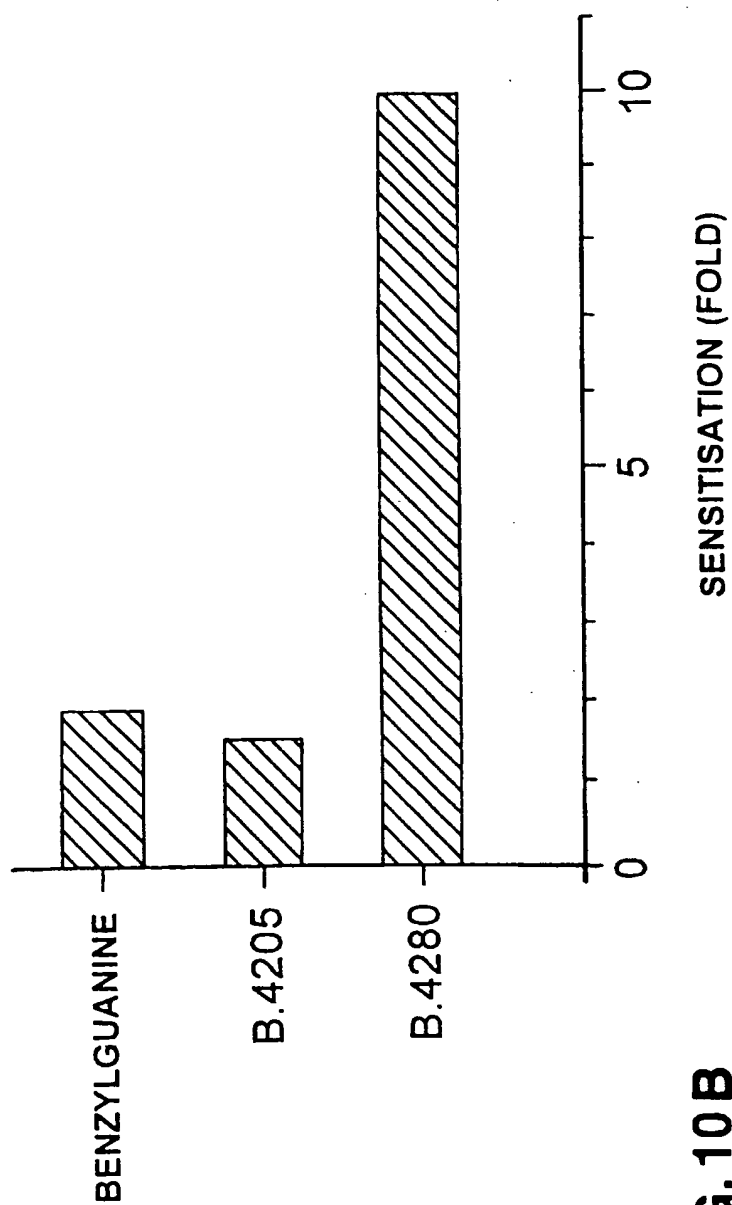


FIG. 10 B

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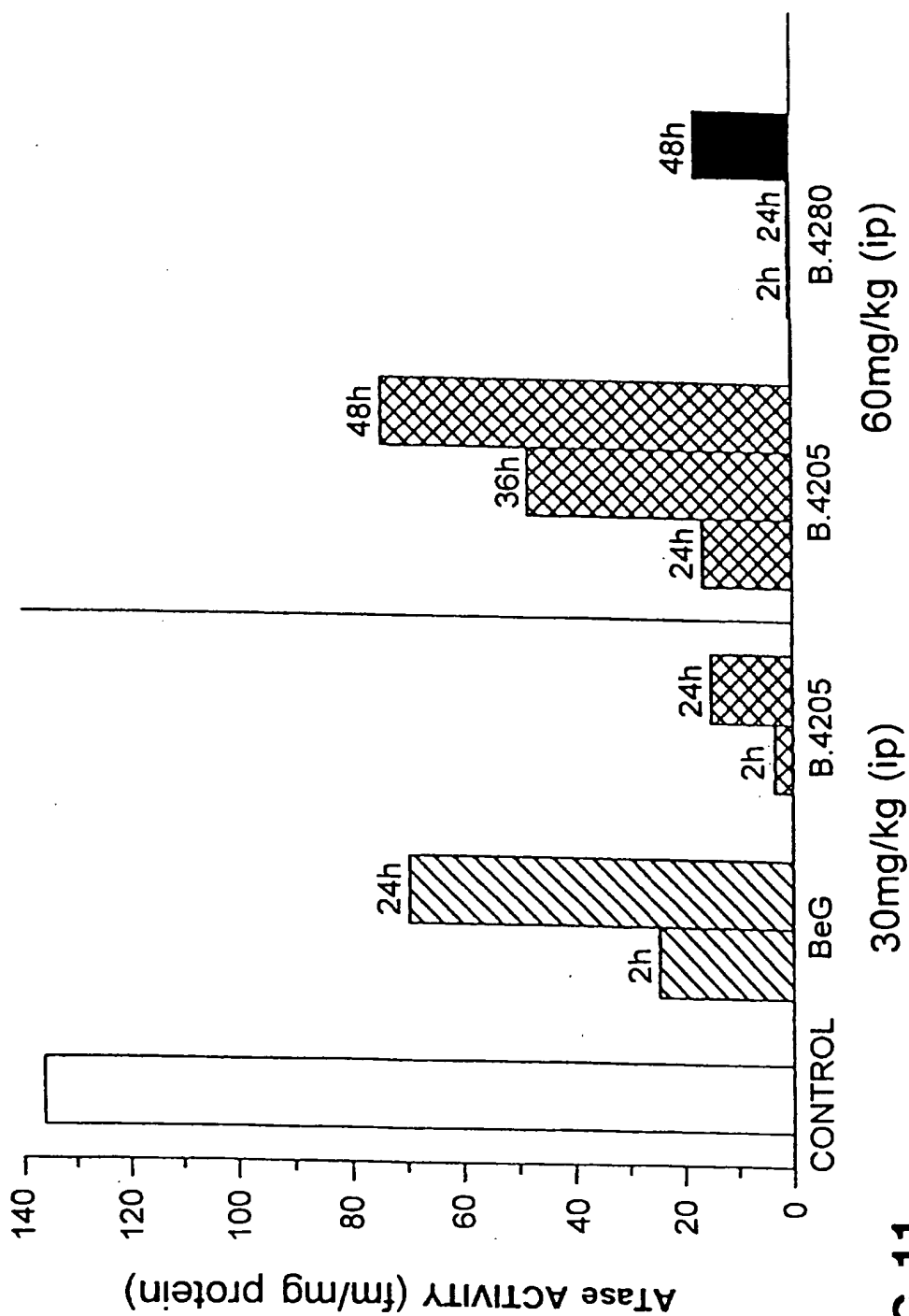
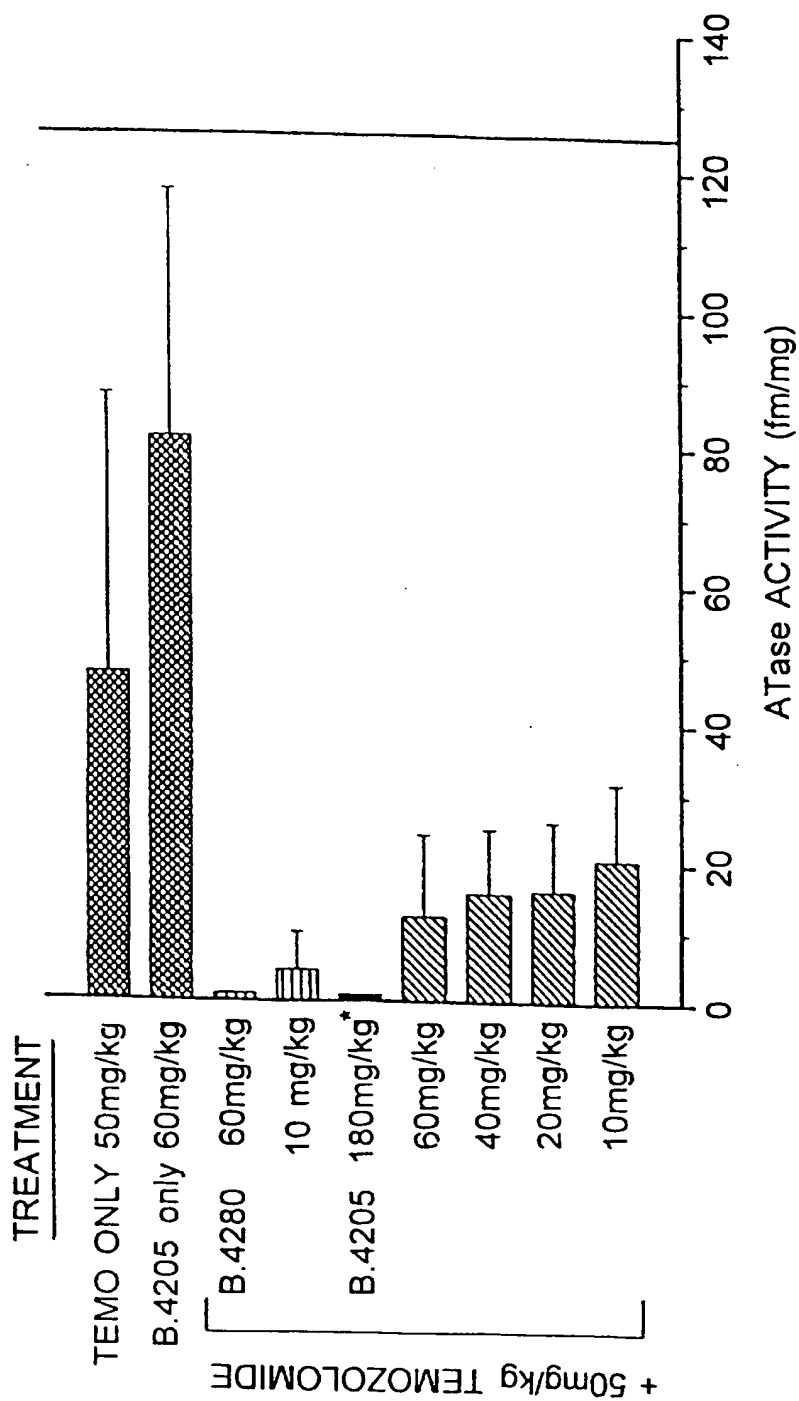


FIG. 11

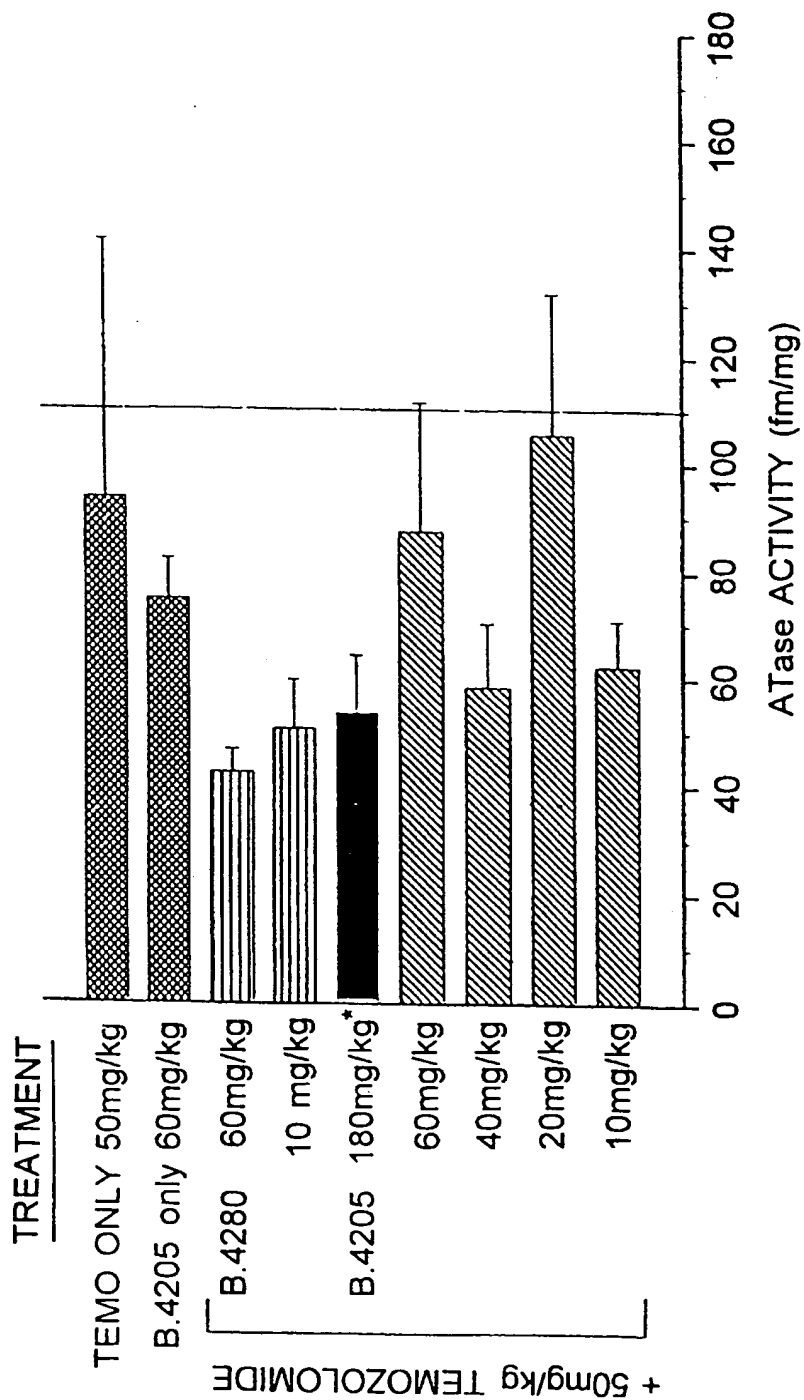
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*180 mg/kg B.4205 + 50mg/kg TEMOZOLOMIDE GIVEN ON DAY 1.
FOLLOWED BY 50mg/kg TEMOZOLOMIDE ONLY ON DAYS 2 + 3

FIG. 12

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*180 mg/kg B.4205 + 50mg/kg TEMOZOLOMIDE GIVEN ON DAY 1.
FOLLOWED BY 50mg/kg TEMOZOLOMIDE ONLY ON DAYS 2 + 3

FIG. 13

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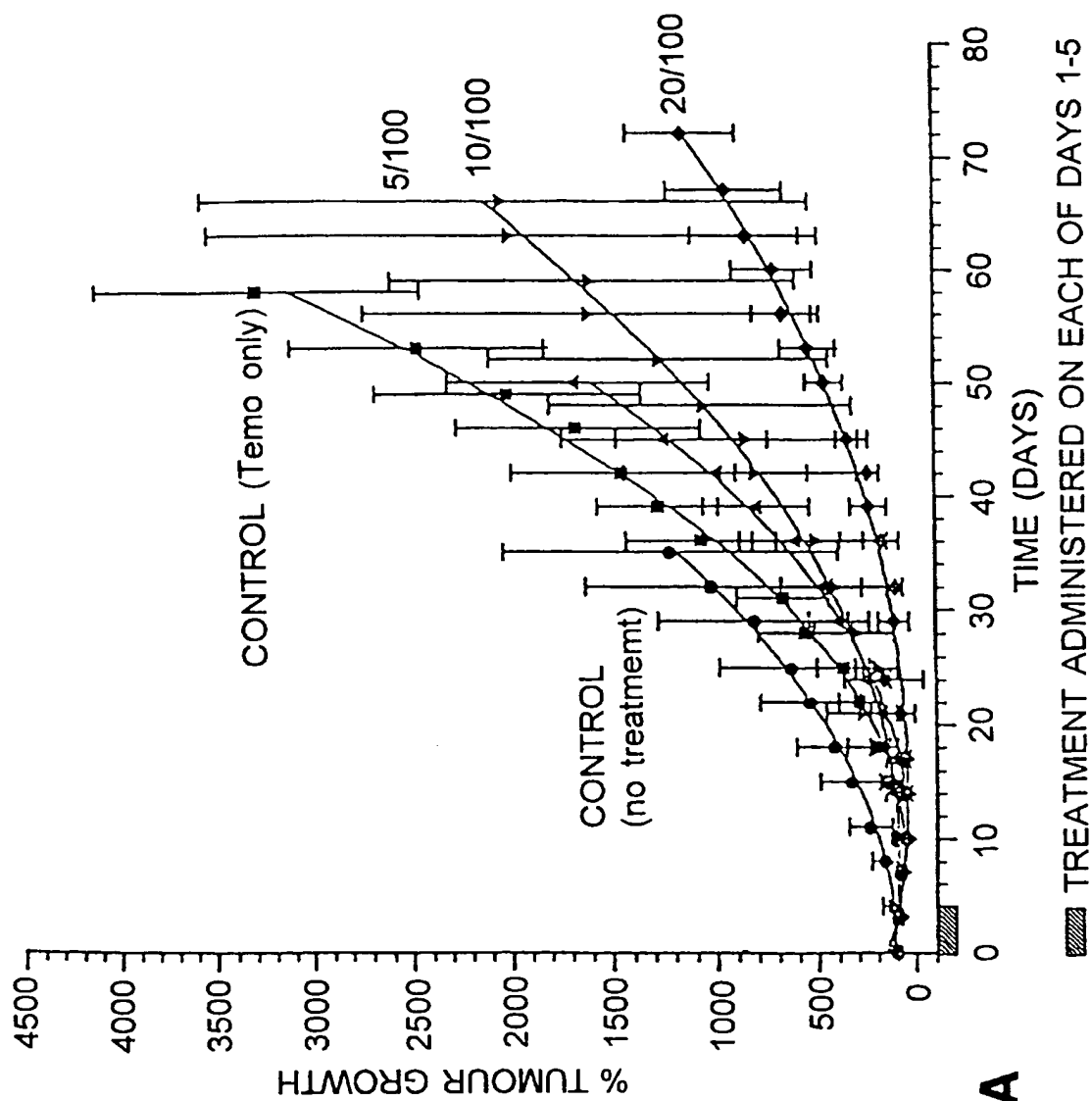


FIG. 14 A

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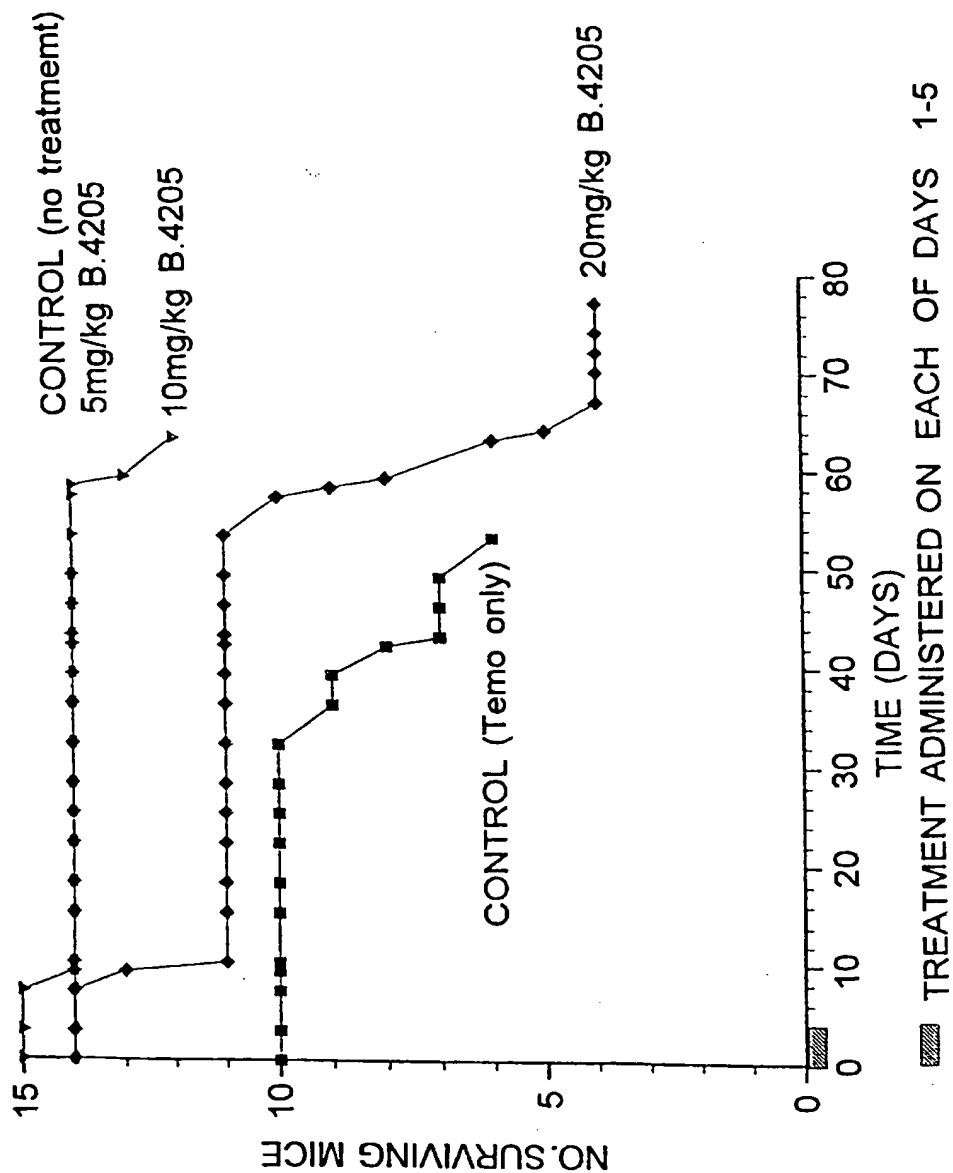
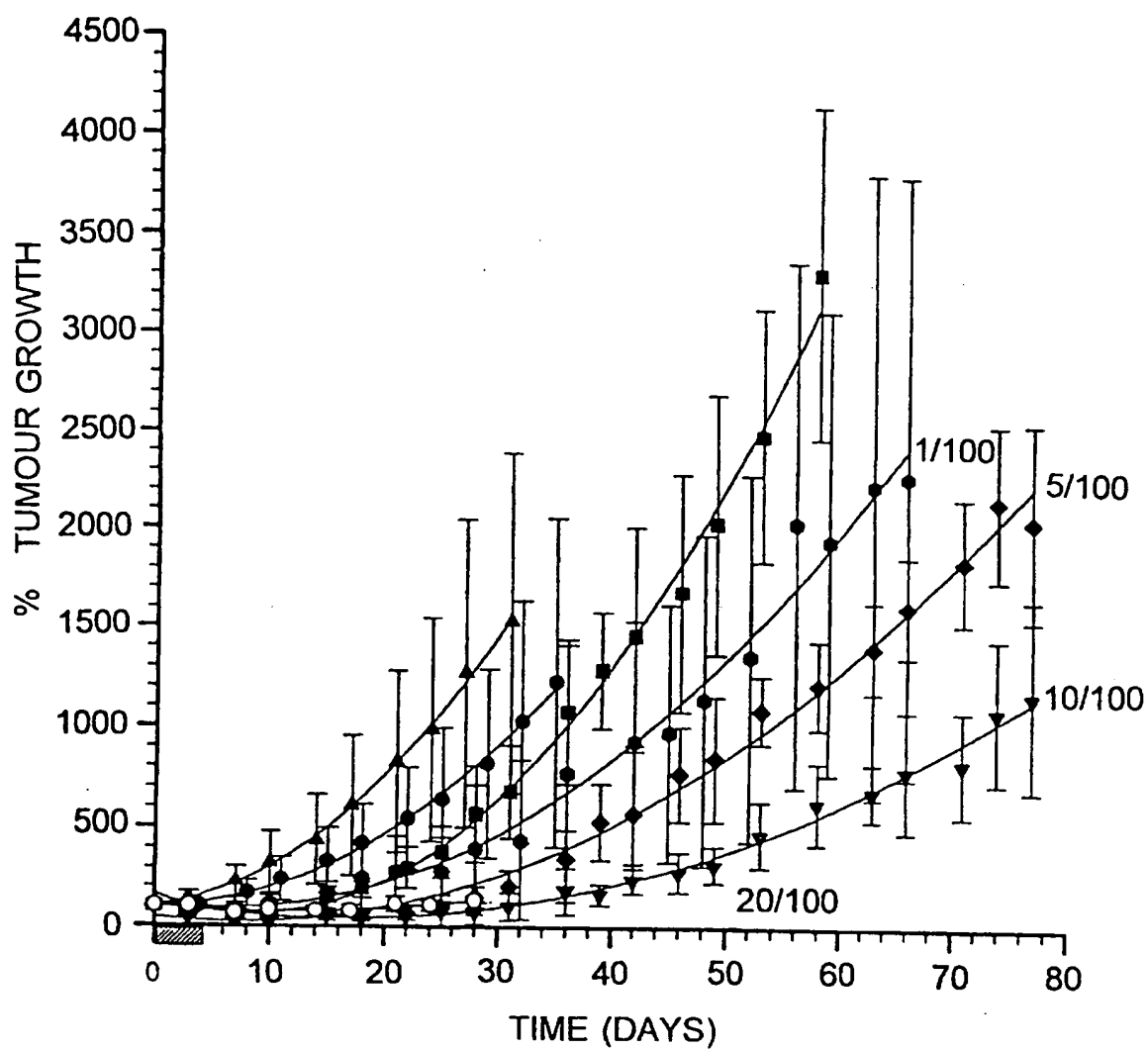


FIG. 14B

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■ TREATMENT ADMINISTERED ON THESE DAYS

▲ CONTROL (20mg/kg B4280)

● CONTROL (no treatment)

■ CONTROL (Temo only)

FIG. 15A

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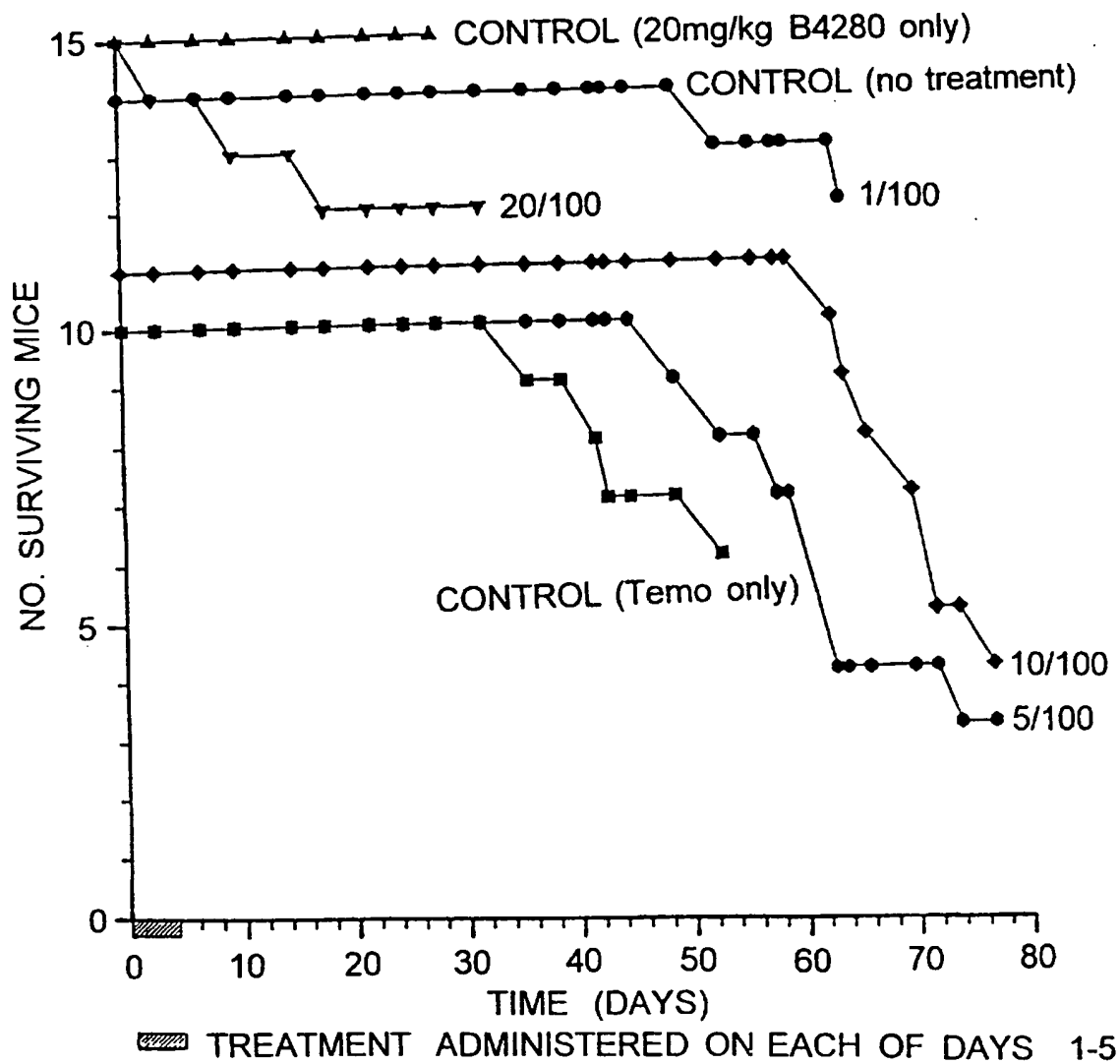
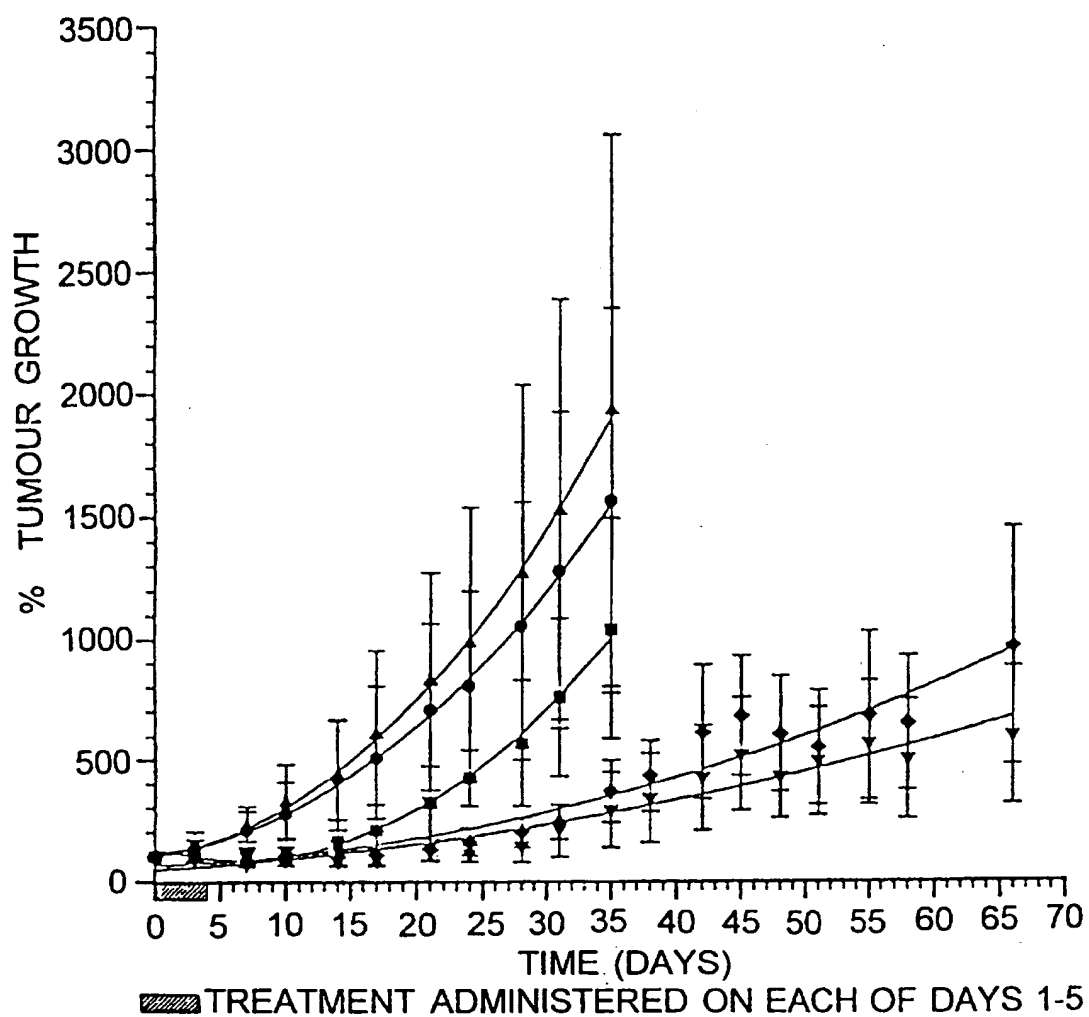


FIG. 15B

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**FIG. 16A**

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- ▲ CONTROL (20mg/kg B. 4280 only)
- CONTROL (no treatment)
- CONTROL (100mg/kg TEMO only)
- ◆ 30mg/kg B.4280 + 100mg/kg TEMO (po)
- ▼ 20mg/kg B.4280 + 100mg/kg TEMO (ip)

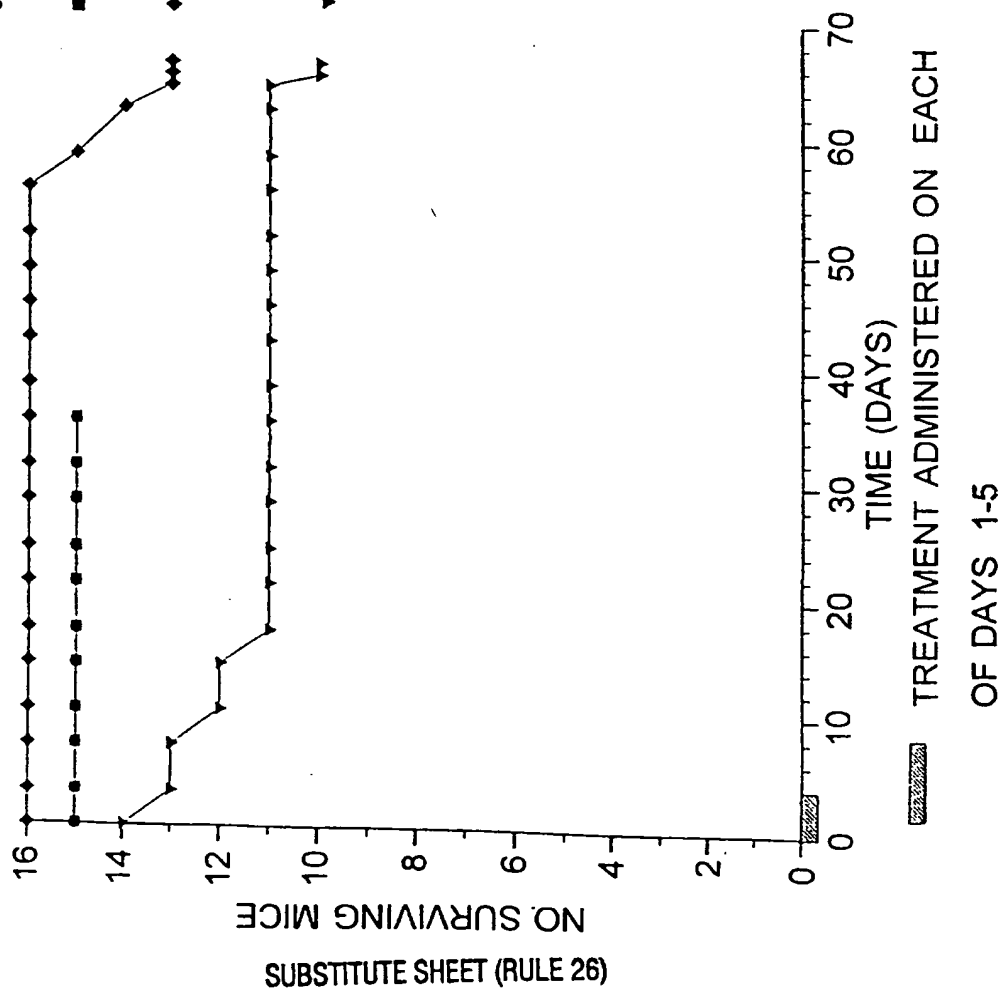


FIG. 16 B

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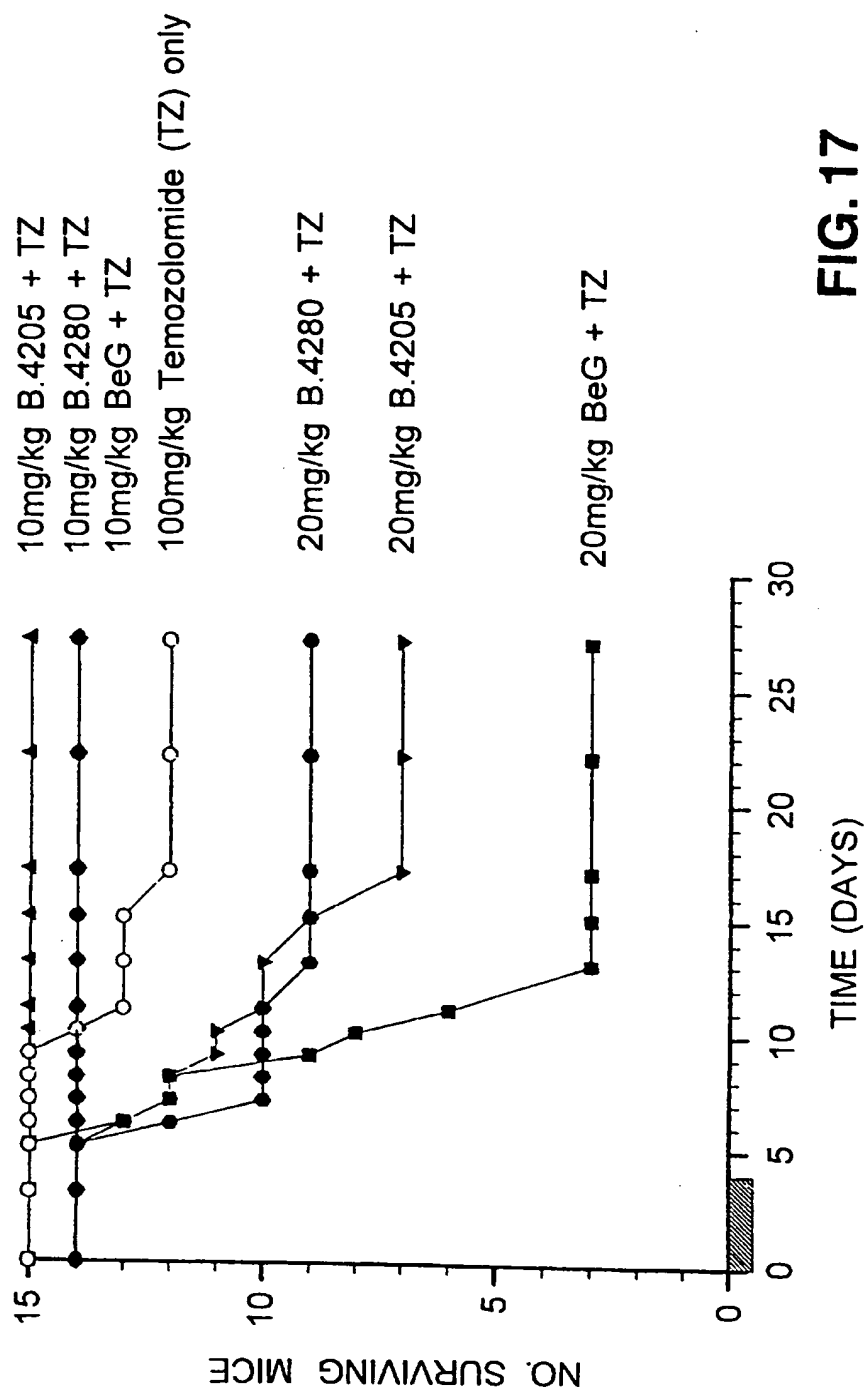


FIG. 17

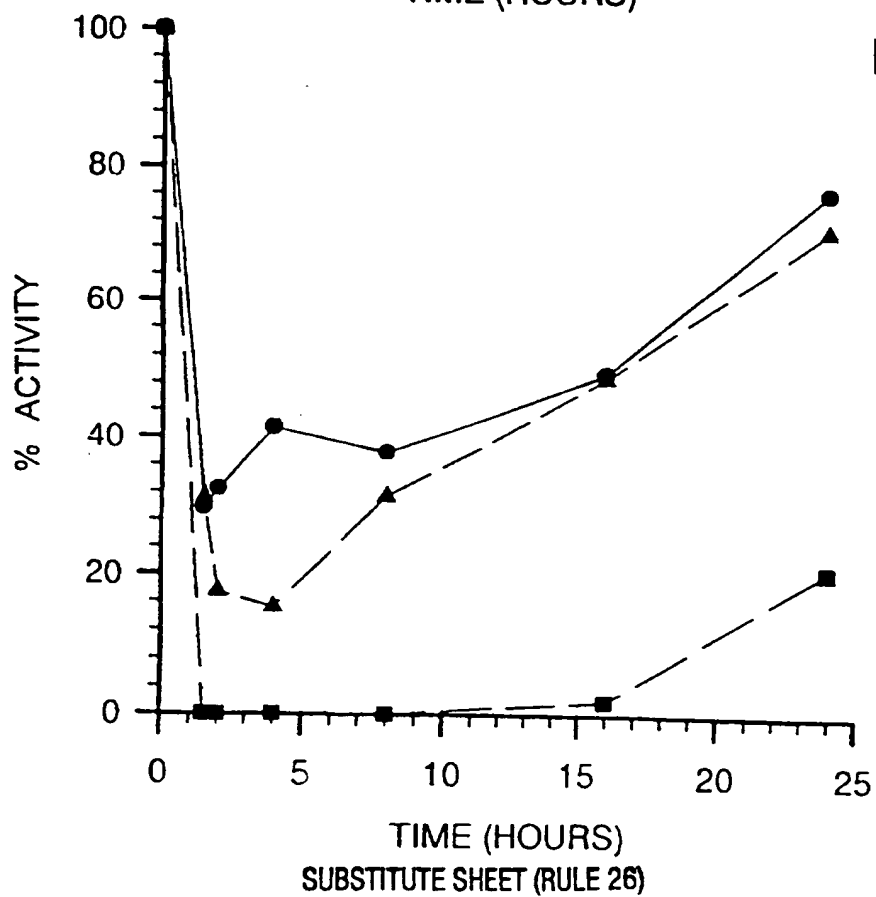
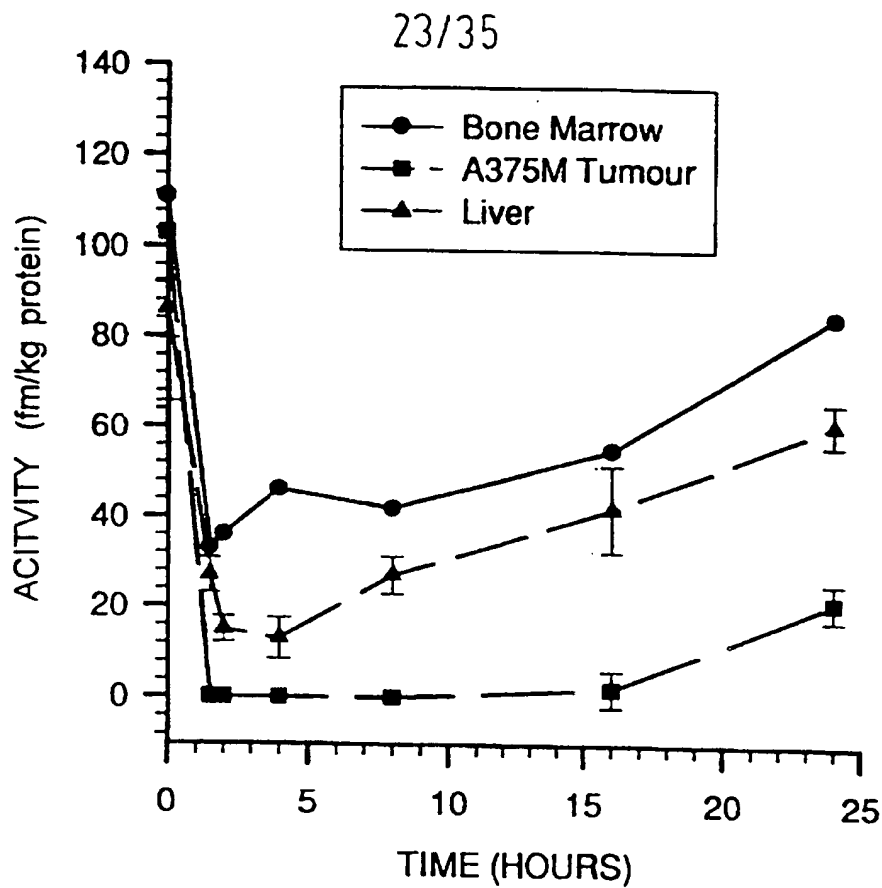
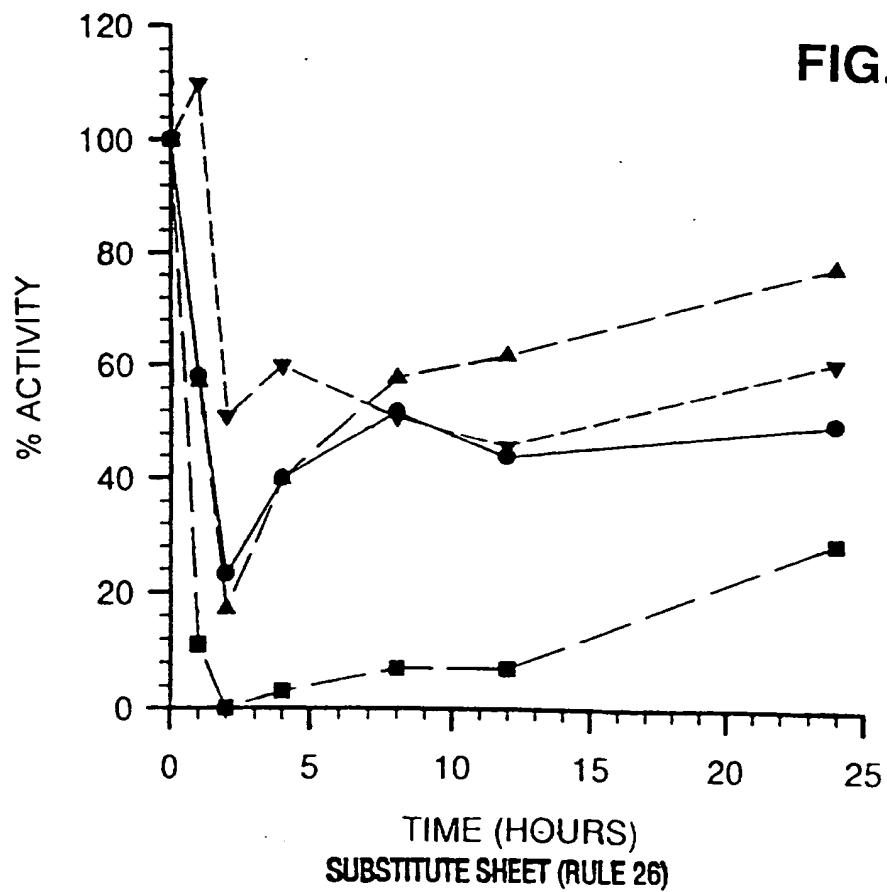
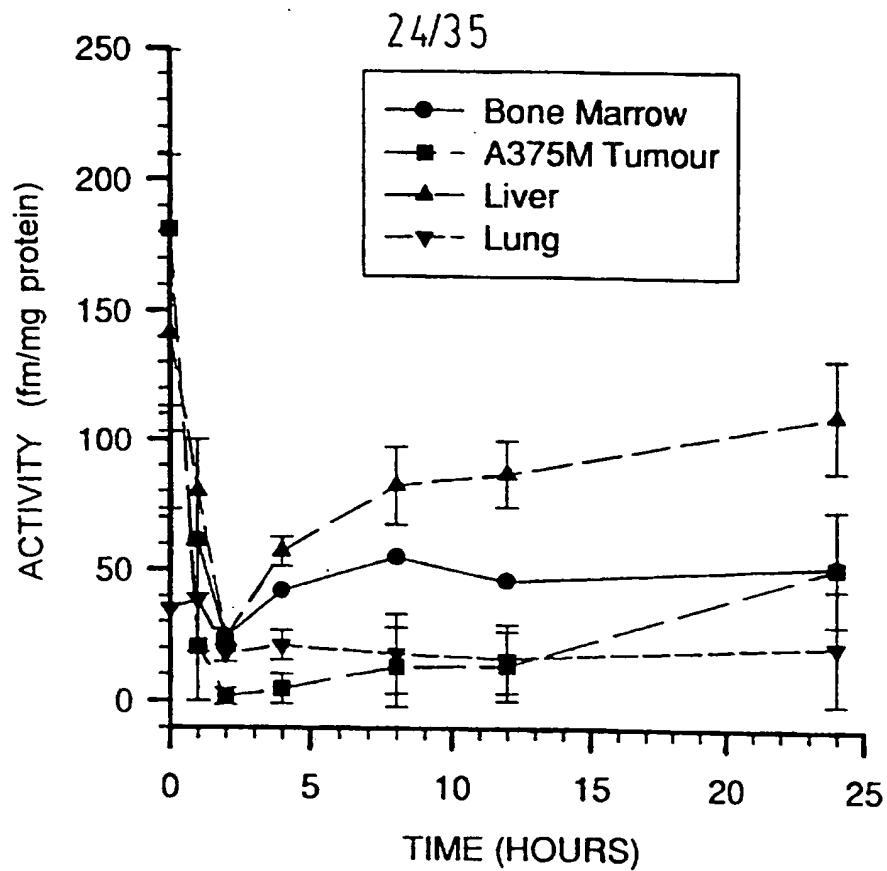


FIG. 18



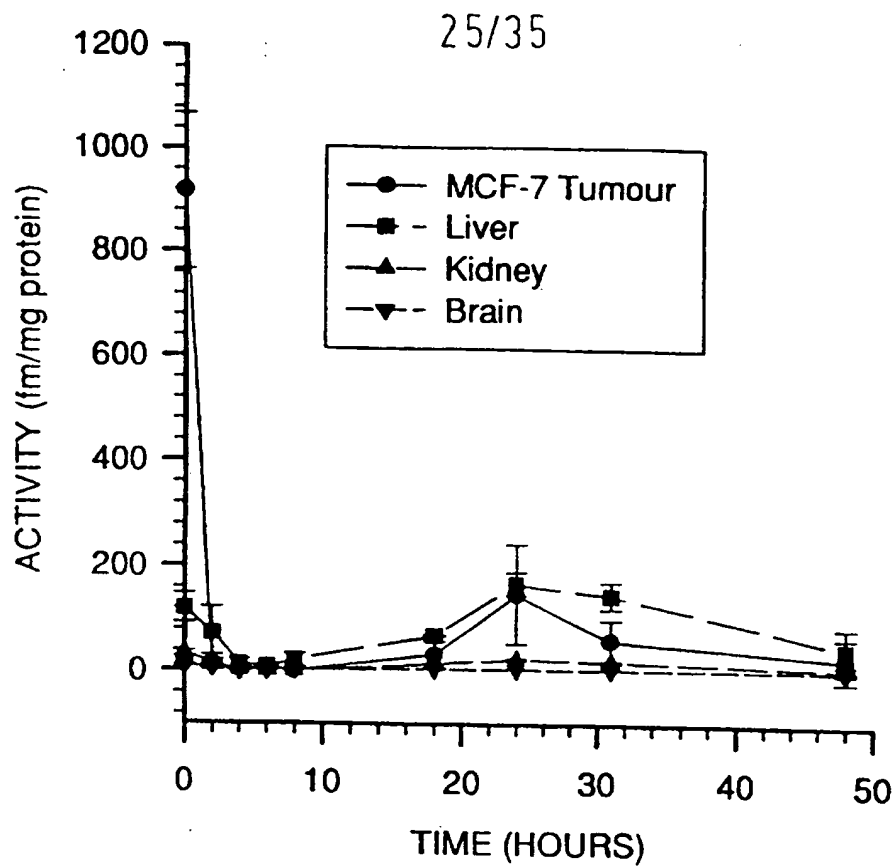
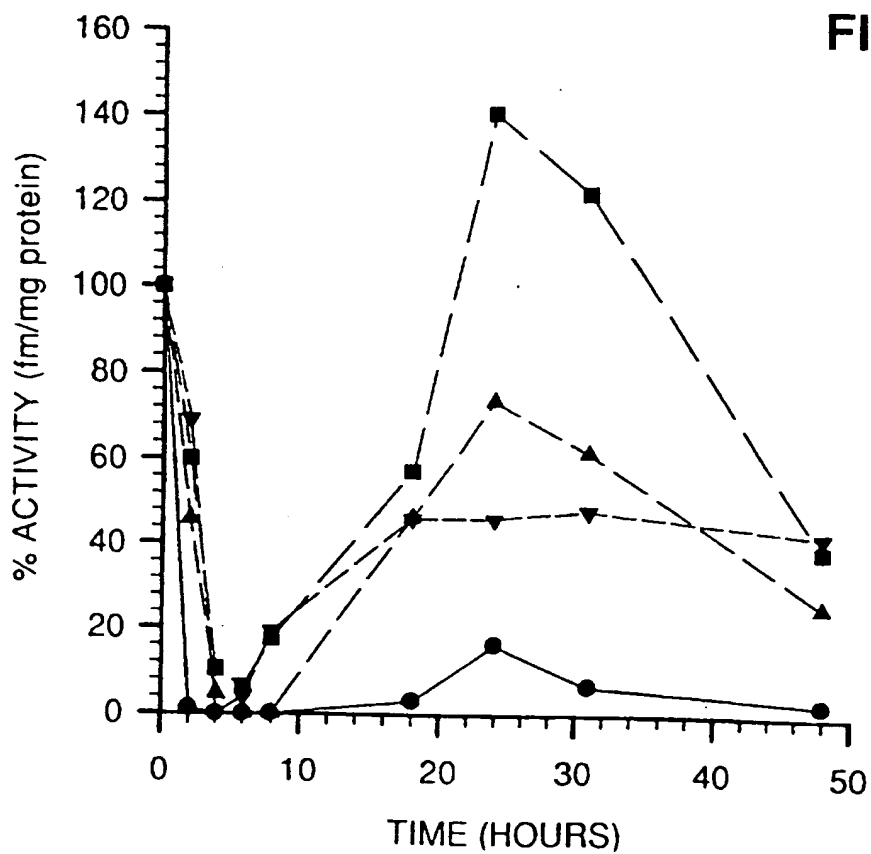


FIG. 20



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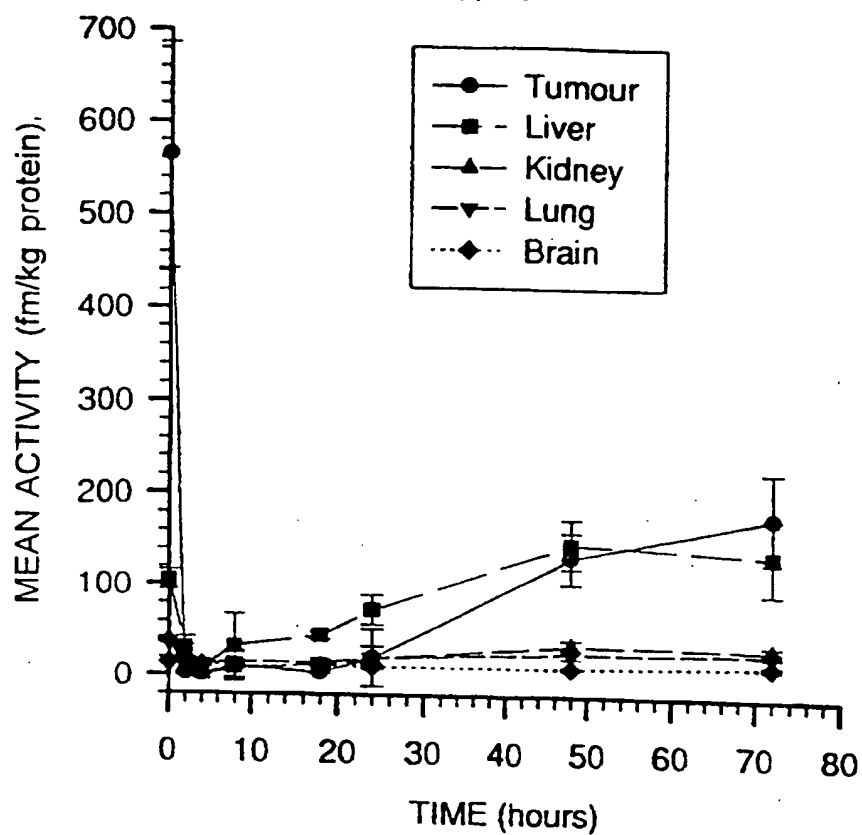
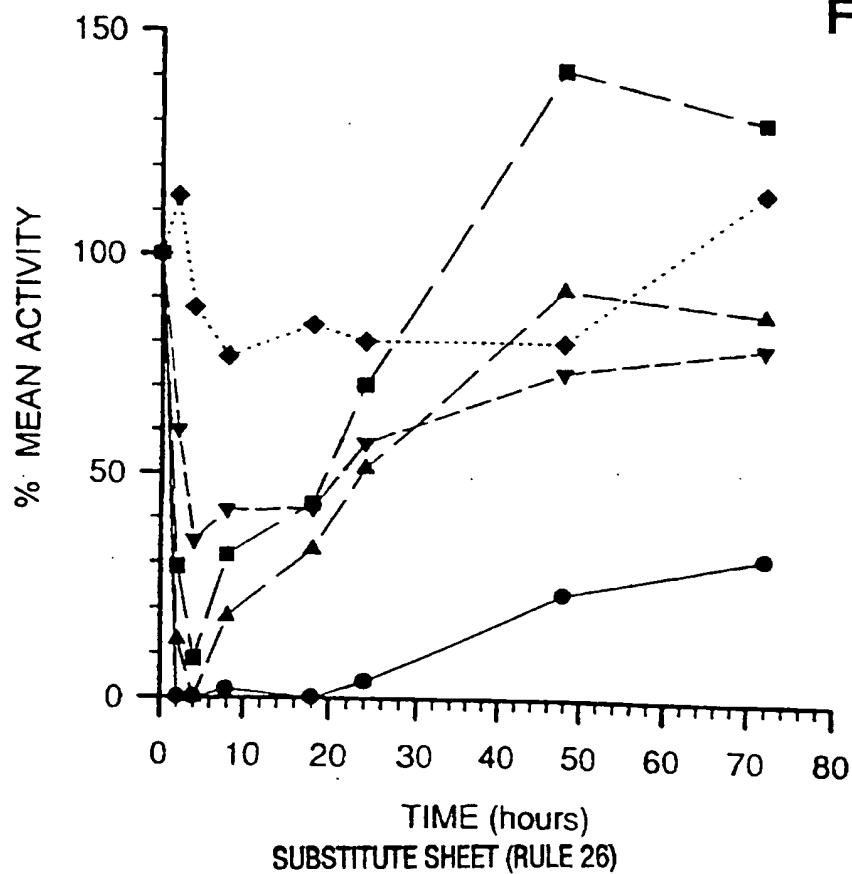


FIG. 21



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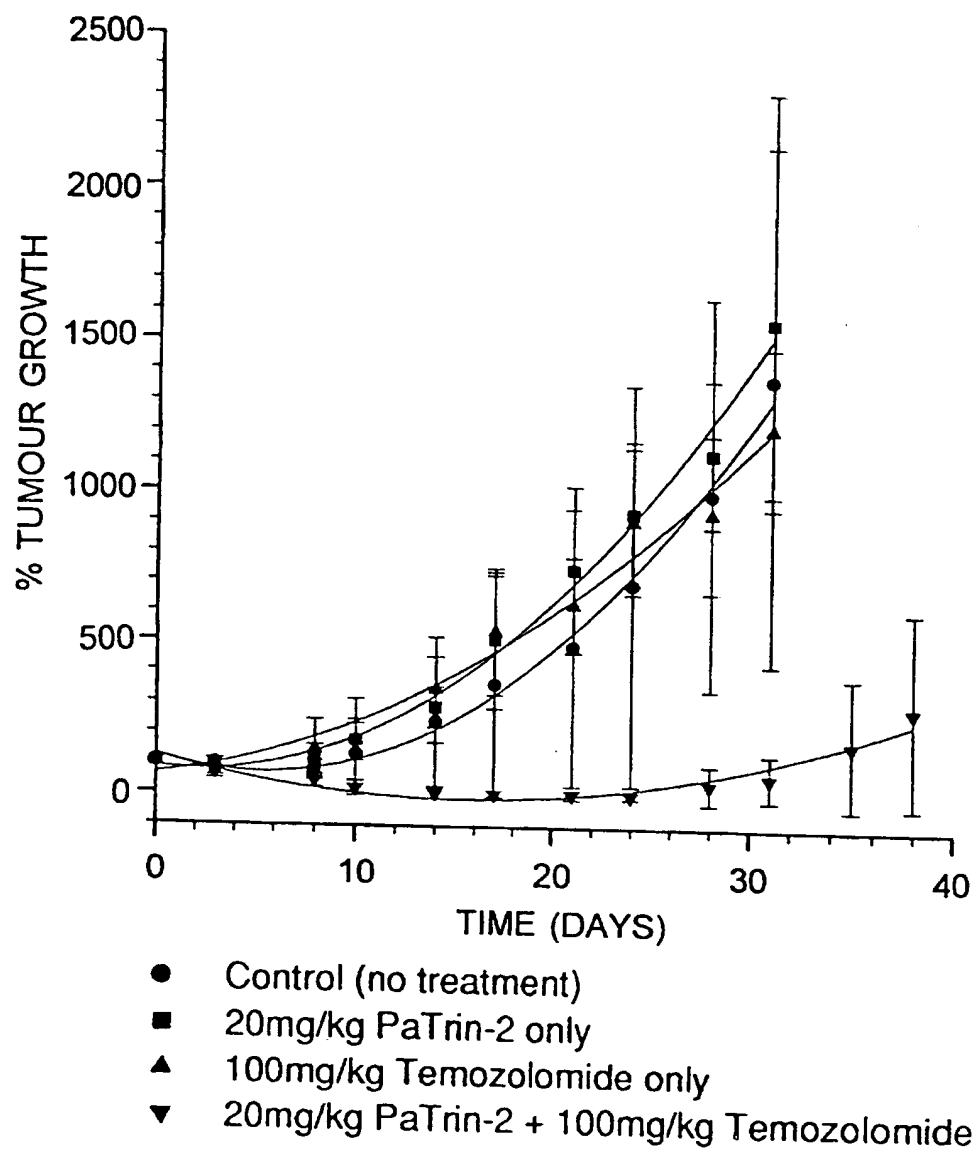
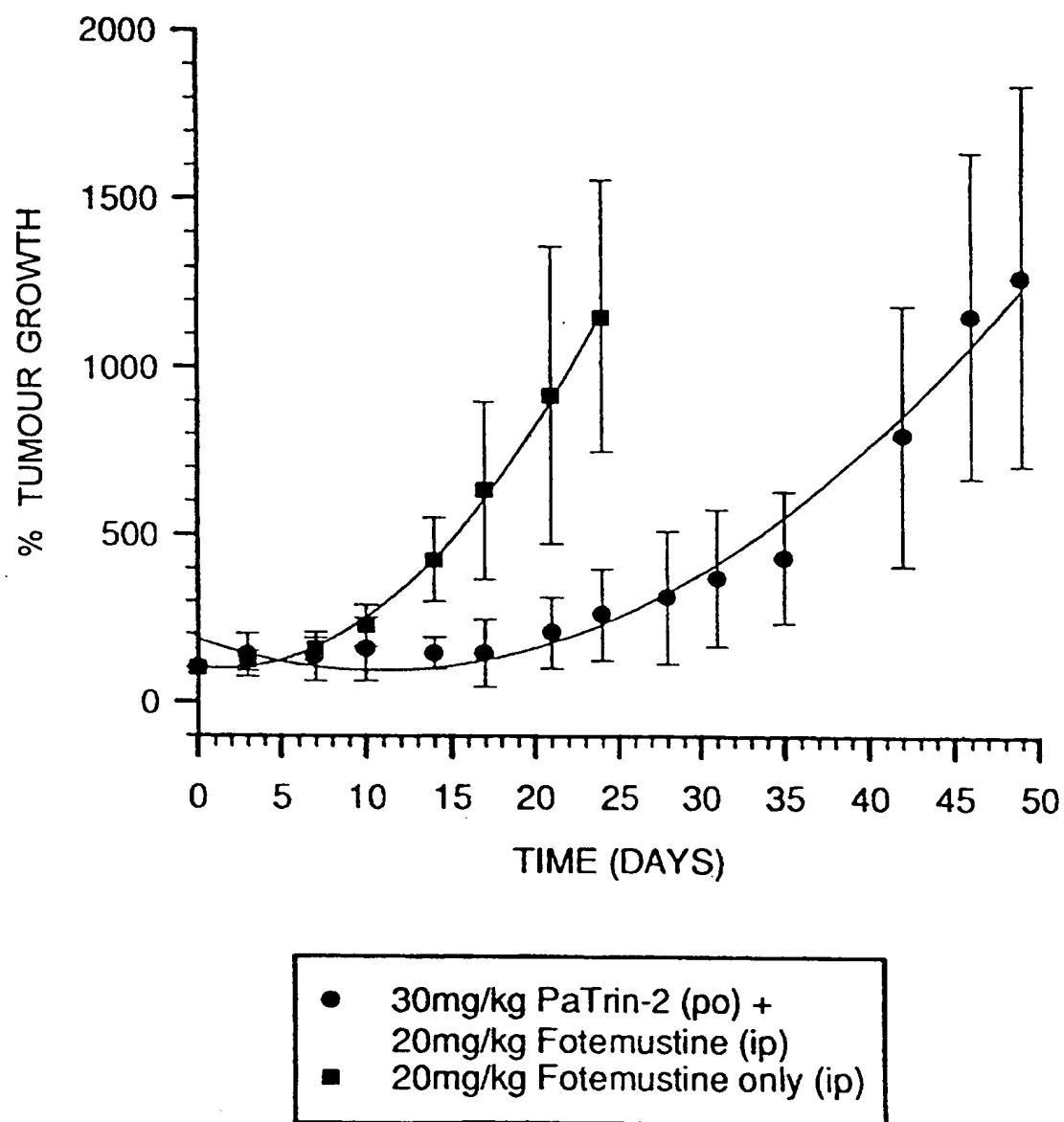


FIG. 22

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**FIG. 23 A**

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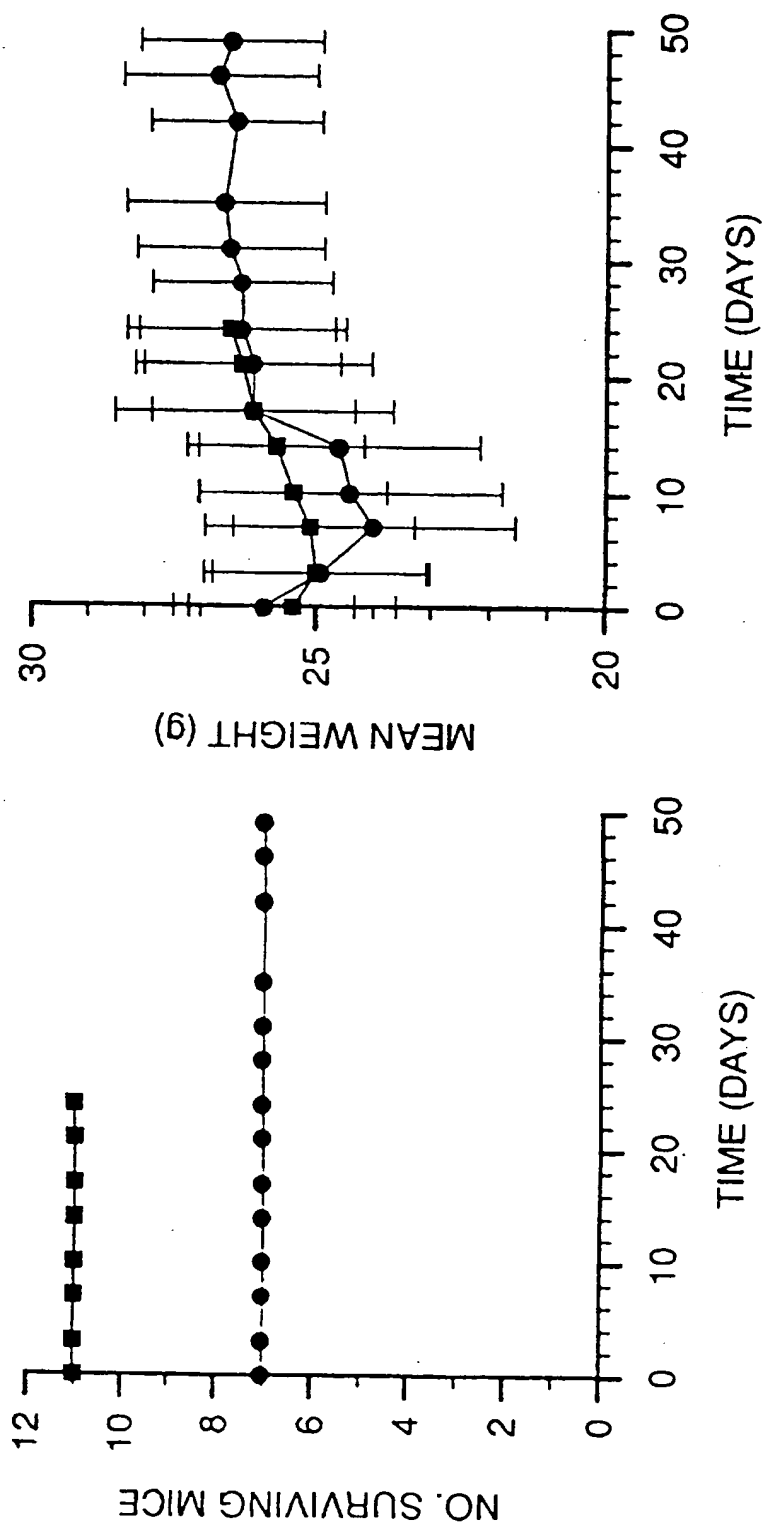


FIG. 23B

- 30mg/kg PaTrin-2 (po) + 20mg/kg Fotemustine (ip)
- 20mg/kg Fotemustine only (ip)

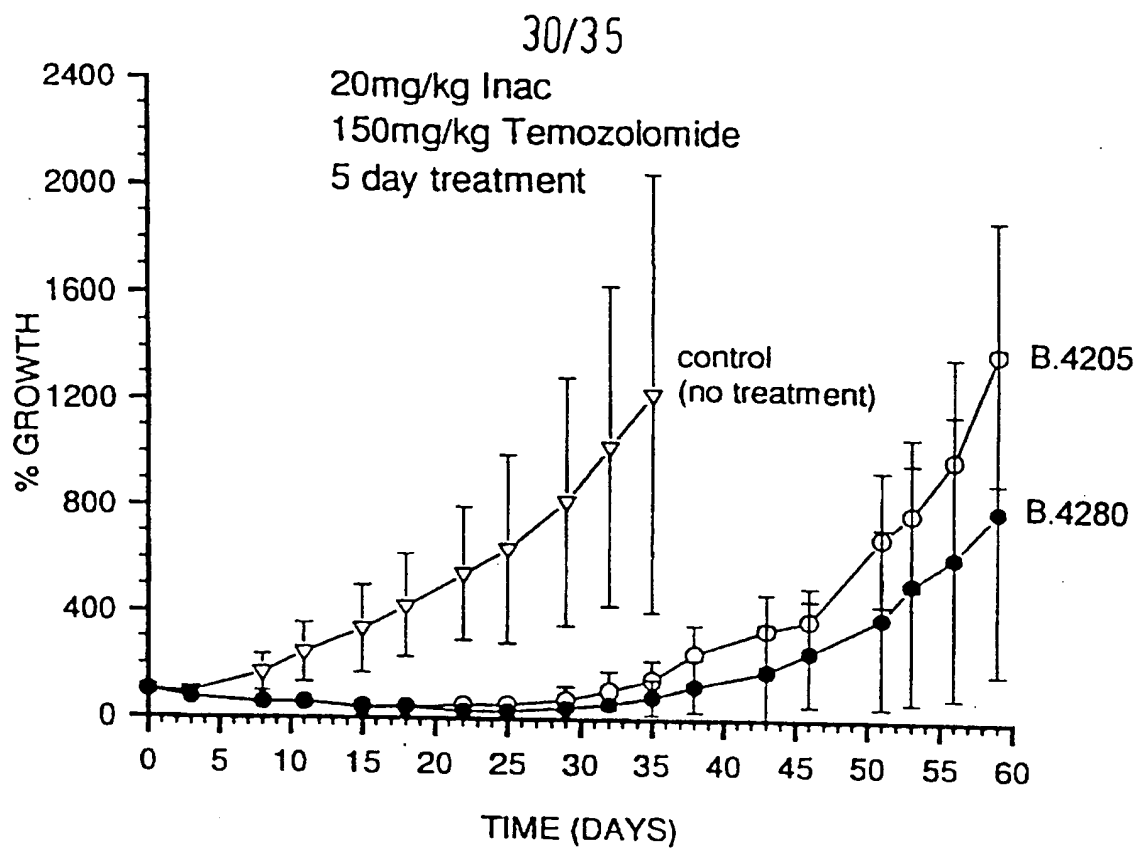
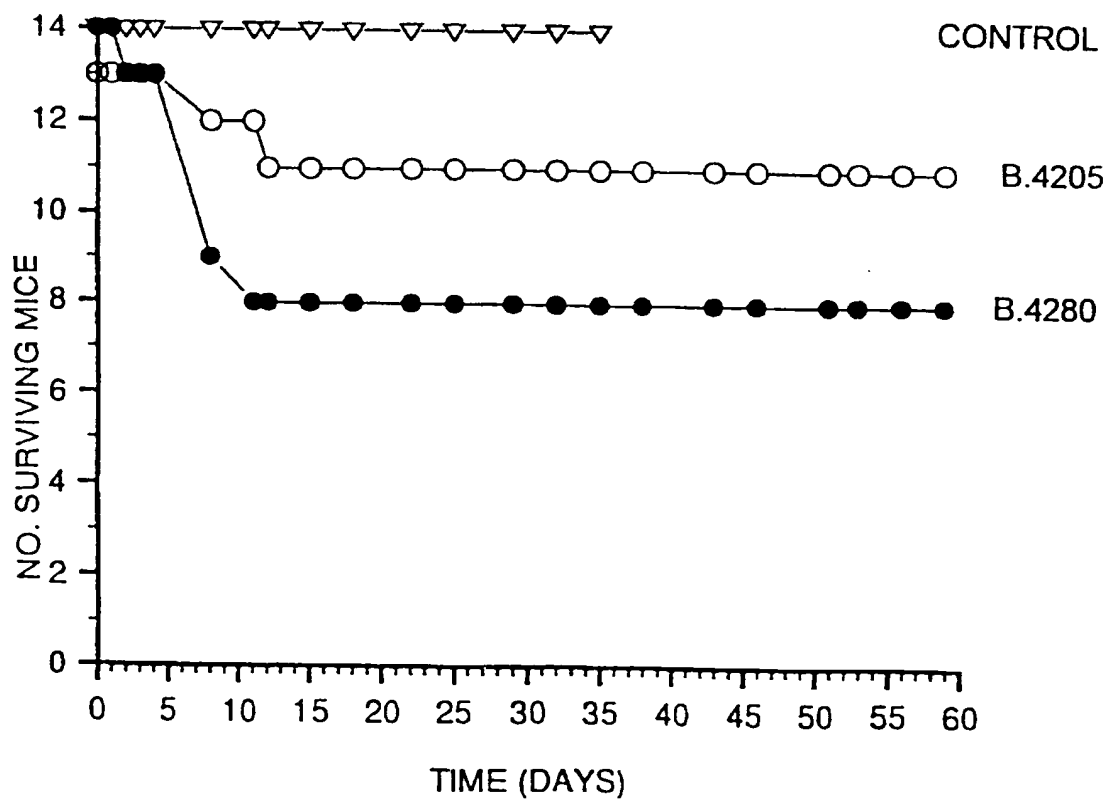


FIG. 24



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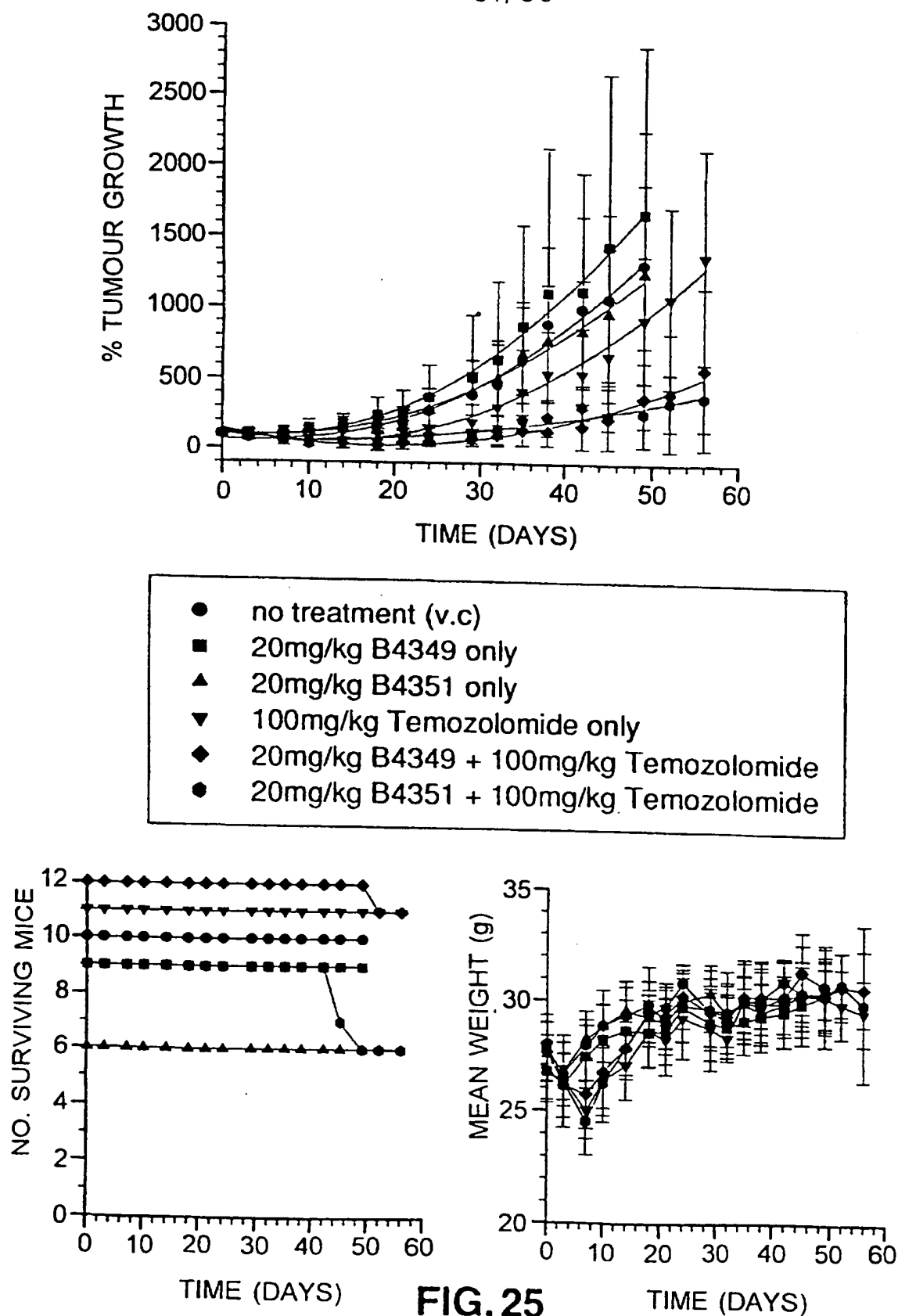


FIG. 25

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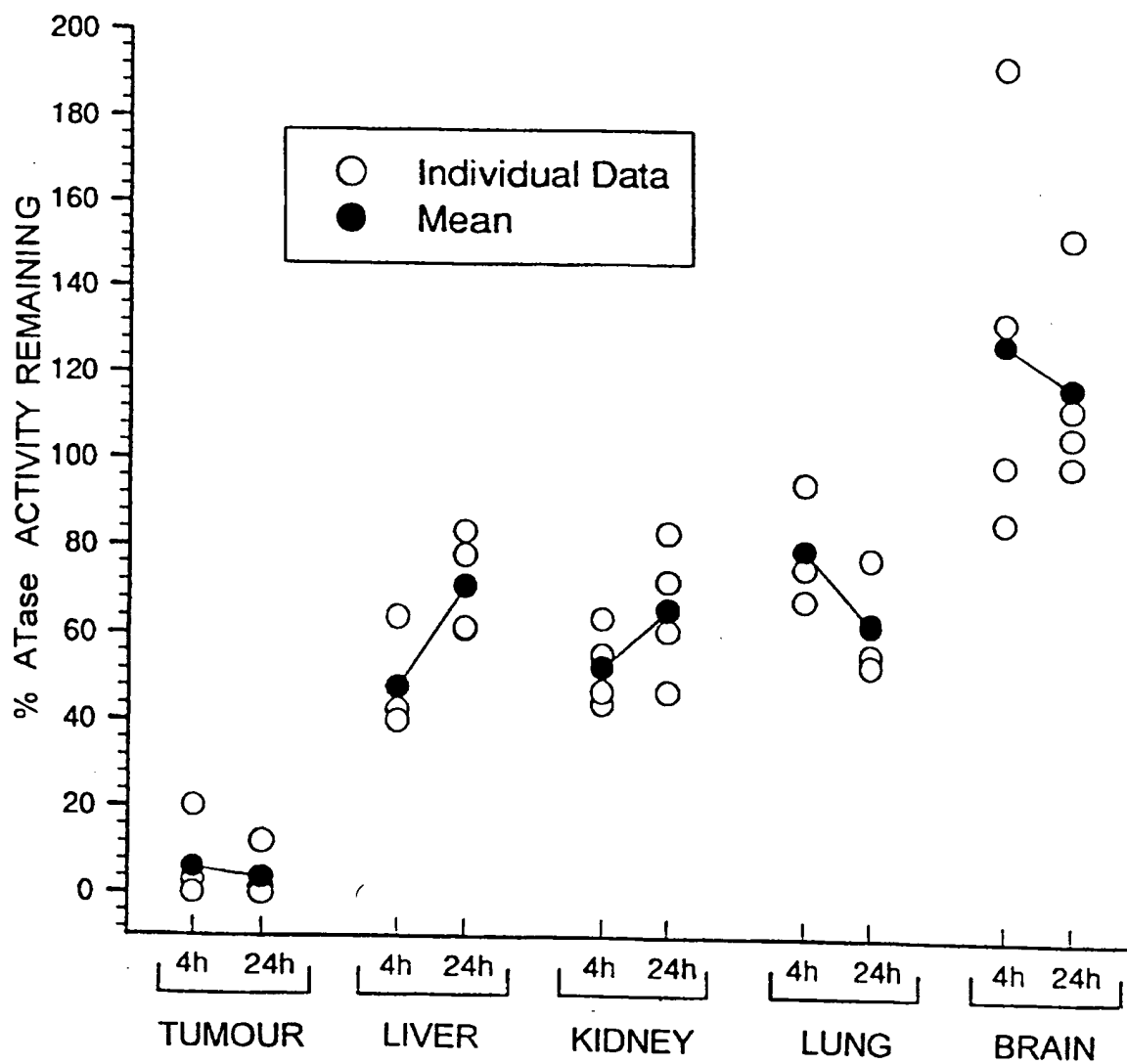


FIG. 26

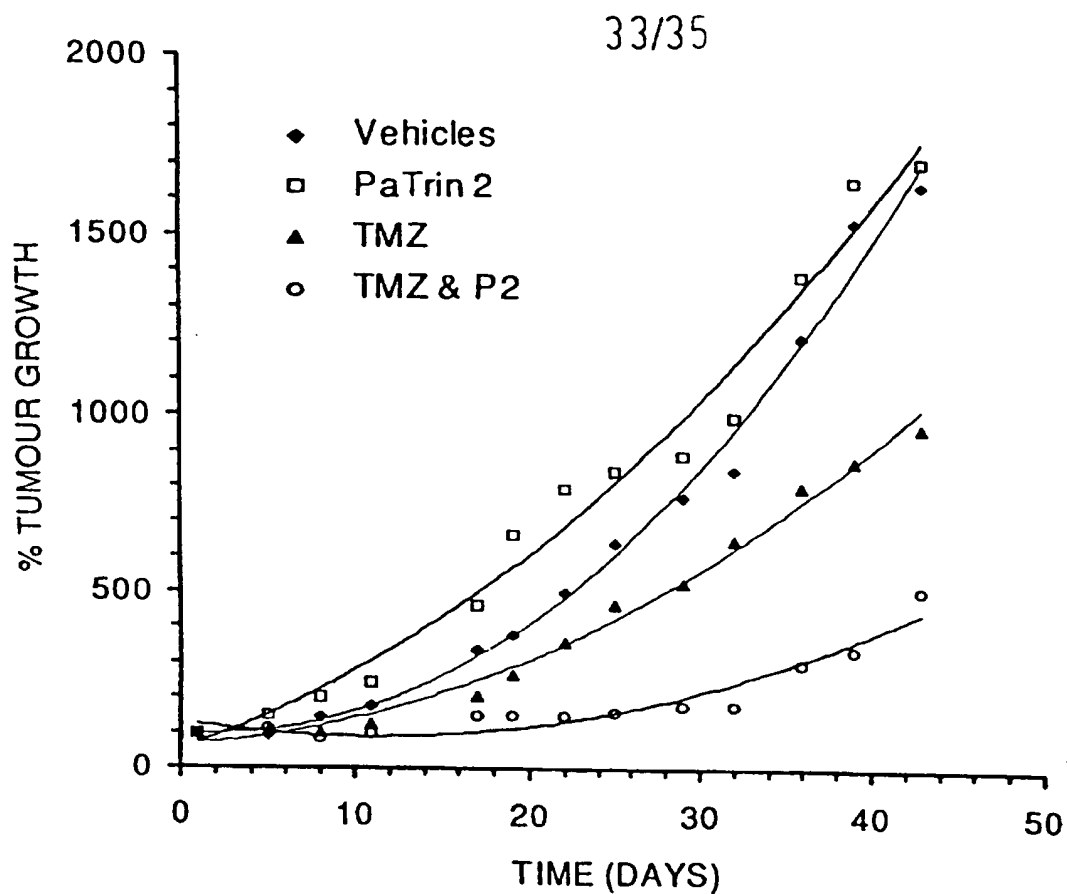
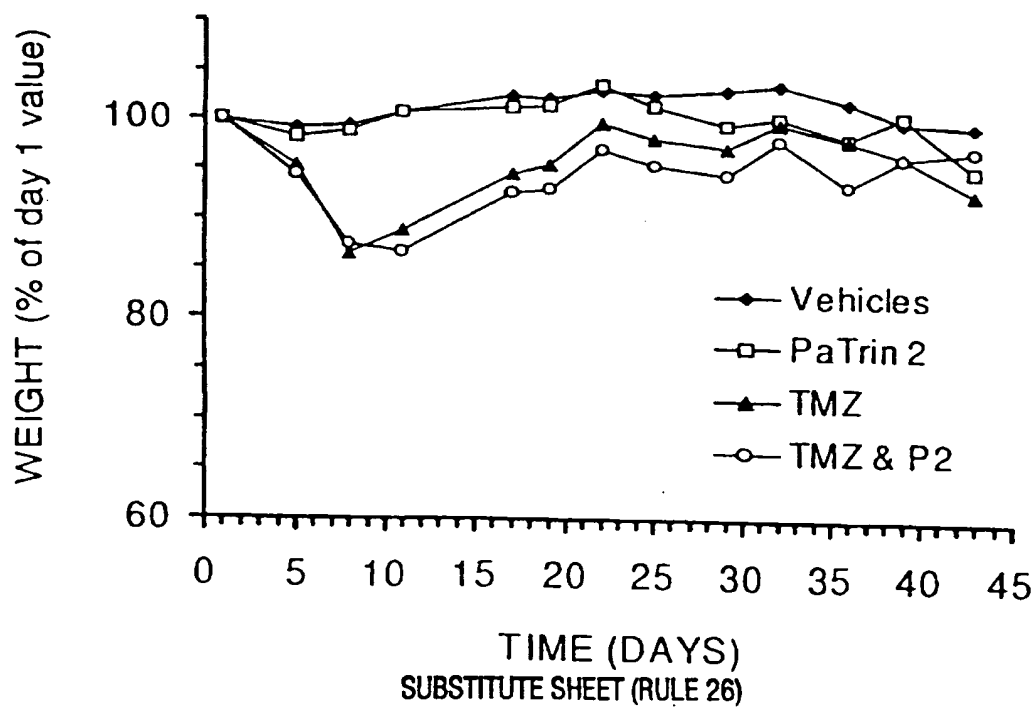
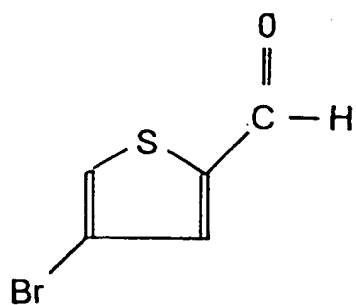


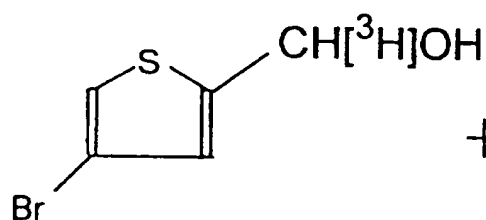
FIG. 27



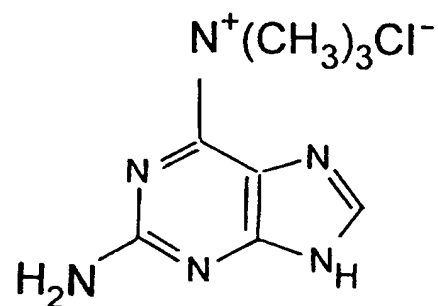
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**FIG. 28**

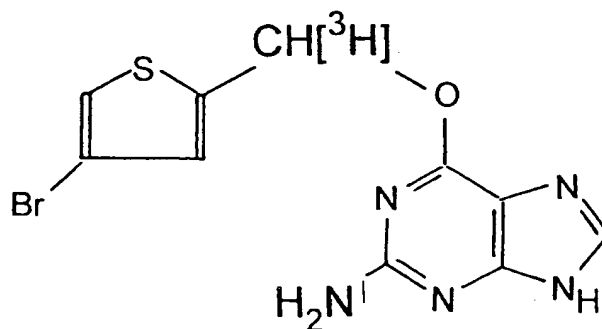
4-BROMO-2- THIOPHENECARBOXALDEHYDE

 $+ \text{NaB}[\text{}^3\text{H}]_4$ 

+

 $[\text{}^3\text{H}]$ 4-BROMOTHENYLALCOHOL

GUANINE SALT

 $+ \text{NaH}$  $[\text{}^3\text{H}]$ 4- BROMOTHENYLGUANINE
SUBSTITUTE SHEET (RULE 26)

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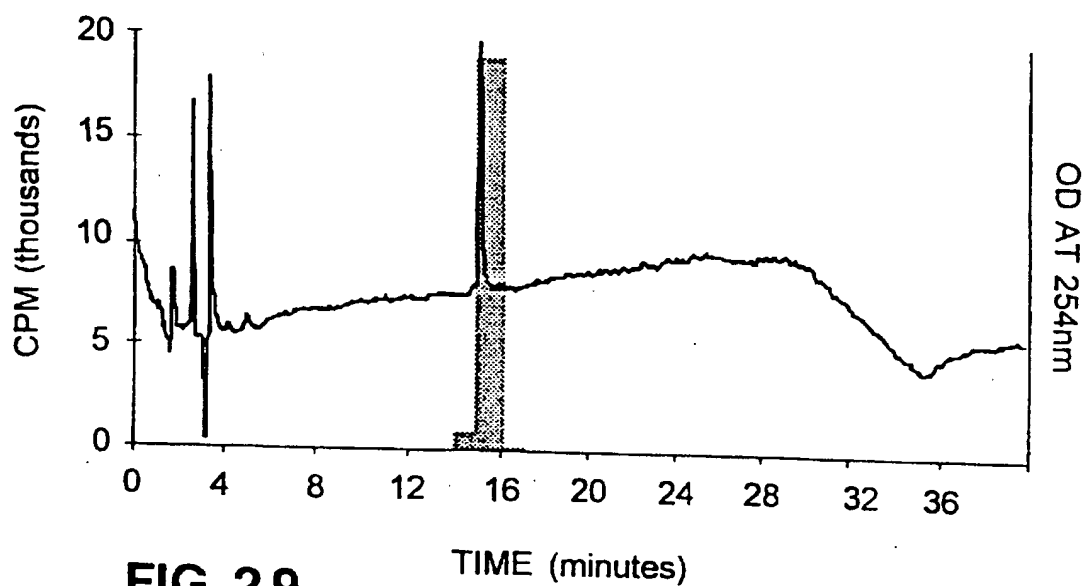


FIG. 29

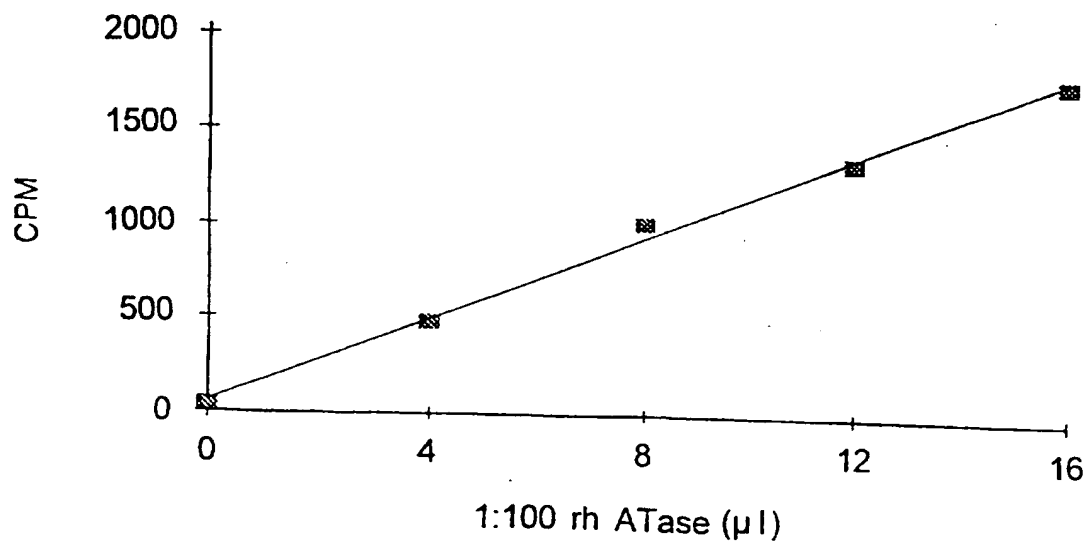


FIG. 30

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IE 96/00084

| | | | | | |
|---|--|--|--|--|-----------------------|
| A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C07D473/18 C07D473/40 C07D251/52 A61K31/52 A61K31/505 C07D473/22 C07D239/30 C07H19/16 C07D498/04 C07D513/04 C07D471/04 C07D475/02 C07D409/12 C07D487/04 | | | | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | | | | |
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C07D C07H | | | | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | | | | |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used) | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | |
| Category | Citation of document, with indication, where appropriate, of the relevant passages | | | | Relevant to claim No. |
| 6 | A CHEMICAL ABSTRACTS, vol. 101, no. 25, 17 December 1984 Columbus, Ohio, US; abstract no. 230466q, page 765; column R; XP002028572 see abstract & HETEROCYCLES, vol. 22, no. 8, 1984, pages 1789-1790, RAM SIYA ET AL: | | | | 2 |
| 6 | X THE JOURNAL OF ORGANIC CHEMISTRY, vol. 34, no. 7, July 1969, pages 2160-2163, XP002028568 MORRIS J. ROBINS ET AL: Page 2161; column 2: formula 13* <div style="text-align: center;">---</div> <div style="text-align: center;">-/--</div> | | | | 2-43 |
| <div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the copy of this report. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div> | | | | | |
| * Special categories of cited documents: <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>'A' document defining the general state of the art which is not considered to be of particular relevance</p> <p>'B' earlier documents not published up or after the international filing date</p> <p>'L' document which may throw doubts on priority claim(s) or which is cited to establish the prior art of another claim or other special reason (as specified)</p> <p>'O' document referring to an oral disclosure, use, exhibition or other means</p> <p>'P' document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>'I' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>'E' document member of the same patent family</p> </div> </div> | | | | | |
| Date of the communication of the international search <div style="text-align: center; font-weight: bold;">1 April 1997</div> | | | Date of mailing of the international search report <div style="text-align: center; font-weight: bold;">09.04.97</div> | | |
| Name and mailing address of the ISA European Patent Office, P.O. Box 5818 Patentlaan 2 NL-2280 HV Rijswijk T L (+31-70) 340-2040, Tlx 31 651 epo nl, F L (+31-70) 340-1016 | | | Authorized officer <div style="text-align: center; font-weight: bold;">Luyten, H</div> | | |

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IE96/00084

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 // (C07D487/04, 249:00, 239:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Publication of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| 1 X | JOURNAL OF MEDICINAL CHEMISTRY, vol. 38, no. 2, 20 January 1995, pages 359-365, XP002028569 MI-YOUNG CHAE ET AL: cited in the application --- | 2-43 |
| 1 X | JOURNAL OF MEDICINAL CHEMISTRY, vol. 37, no. 3, 4 February 1994, pages 342-347, XP002028570 MI-YOUNG CHAE ET AL: cited in the application *Article* --- -/-- | 2-43 |

☒ Further documents are listed in the continuation of box C.

☐ Patent (family members are listed in annex).

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *I* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *U* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *A* document member of the same patent family

Date of the latest completion of the international search

Date of mailing of the international search report

Name and mailing address of the ISA
European Patent Office, P.O. Box 1201, 6000 Luxembourg
N... 2280 11V Rijswijk
T: (+31-70) 340-2040, Tx: 31 651 epo nl,
F: (+31-70) 340-3016

Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IE96/00084

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|--|-----------------------|
| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| 1 X | JOURNAL OF MEDICINAL CHEMISTRY, vol. 35, no. 23, 13 November 1992, pages 4486-4491, XP002028571 ROBERT C. MOSCHEL ET AL: cited in the application *Article* | 1.2 |
| 1 X | --- WO 91 13898 A (THE UNITED STATES OF AMERICA) 19 September 1991 cited in the application see page 28 - page 32; claims | 2-43 |
| 1 X | --- WO 94 29312 A (CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED) 22 December 1994 cited in the application see the whole document | 2-43 |
| 1 P,X | --- WO 96 04281 A (THE UNITED STATES OF AMERICA) 15 February 1996 cited in the application see the whole document ----- | 2-43 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IE 96/00084

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 35-36 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IE 96/00084

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
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| | | US 5352669 A | 04-10-94 |
| | | AT 141925 T | 15-09-96 |
| | | AU 646452 B | 24-02-94 |
| | | AU 7582191 A | 10-10-91 |
| | | CA 2078129 A | 14-09-91 |
| | | DE 69121706 D | 02-10-96 |
| | | DE 69121706 T | 23-01-97 |
| | | EP 0523100 A | 20-01-93 |
| | | ES 2091322 T | 01-11-96 |
| | | JP 5504972 T | 29-07-93 |
| | | US 5358952 A | 25-10-94 |
| | | | |
| WO 9429312 A | 22-12-94 | AU 6805994 A | 03-01-95 |
| | | CZ 9503233 A | 12-06-96 |
| | | EP 0702683 A | 27-03-96 |
| | | FI 955906 A | 02-02-96 |
| | | IE 62443 B | 08-02-95 |
| | | JP 8511773 T | 10-12-96 |
| | | NO 954985 A | 07-02-96 |
| | | PL 311950 A | 18-03-96 |
| | | SK 154795 A | 03-07-96 |
| | | ZA 9404026 A | 06-02-95 |
| | | | |
| WO 9604281 A | 15-02-96 | US 5525606 A | 11-06-96 |
| | | AU 3207995 A | 04-03-96 |
| | | CA 2195856 A | 15-02-96 |
| | | | |